



JC17 Rec'd PCT/PTO 15 JUN  
6-18-01

FORM PTO-100  
(REV 10-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

**TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371**

4951US

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

**09/868732**

INTERNATIONAL APPLICATION NO.  
PCT/EP99/10209

INTERNATIONAL FILING DATE  
16 December 1999 (16.12.99)

PRIORITY DATE CLAIMED  
16 December 1998 (16.12.98)

TITLE OF INVENTION

**SELECTING ANIMALS FOR PARENTALLY IMPRINTED TRAITS**

APPLICANT(S) FOR DO/EO/US **Leif ANDERSSON, Michel GEORGES, Geert SPINCEMAILLE,  
Carine Danielle Andree NEZER**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

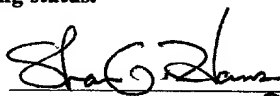
1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
  2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
  3. ☐ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
  4. ☐ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
  5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
    - a. ☒ is attached hereto (required only if not communicated by the International Bureau).
    - b. ☐ has been communicated by the International Bureau.
    - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
  6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
  7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
    - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
    - b. ☐ have been communicated by the International Bureau.
    - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
    - d. ☐ have not been made and will not be made.
  8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
  9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
  10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).
- Items 11 to 16 below concern document(s) or information included:**
11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
  12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
  13. ☒ A **FIRST** preliminary amendment.
  14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
  15. ☐ A substitute specification.
  16. ☐ A change of power of attorney and/or address letter.
  16. ☒ Other items or information:  
Copy of Preliminary Examination Report

**NOTICE OF EXPRESS MAILING**

Express Mail Mailing Label Number: EL740537159US

Date of Deposit with USPS: June 15, 2001

Person making Deposit: Jared Turner

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) <b>097868732</b>		INTERNATIONAL APPLICATION NO. PCT/EP99/10209		ATTORNEY'S DOCKET NUMBER 4951US		
17. <input checked="" type="checkbox"/> The following fees are submitted: <b>BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):</b> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... \$1000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... \$860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$710.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... \$690.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) ..... \$100.00 <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				<b>CALCULATIONS</b> PTO USE ONLY		
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$ 860.00		
				\$ 130.00		
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE			
Total claims	32 - 20 =	12	X \$18.00	\$ 226.00		
Independent claims	3 - 3 =	0	X \$80.00	\$ 0		
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00	\$		
<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$ 1,216.00		
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				\$		
<b>SUBTOTAL =</b>				\$ 1,216.00		
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$		
<b>TOTAL NATIONAL FEE =</b>				\$		
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				\$		
<b>TOTAL FEES ENCLOSED =</b>				\$ 1,216.00		
				Amount to be refunded:	\$	
				charged:	\$	
a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>1,216.00</u> to cover the above fees is enclosed.						
b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.						
c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>20-1469</u> . A duplicate copy of this sheet is enclosed.						
<b>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</b>						
SEND ALL CORRESPONDENCE TO: Allen C. Turner TRASKBRITT, P.C. P.O. Box 2550 Salt Lake City, Utah United States of America						
				 SIGNATURE: <b>FOR</b>	REC. NO. 42,629	
				Allen C. Turner NAME		
				<u>33,041</u> REGISTRATION NUMBER		

**PATENT**  
**4951US**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**In re Application of:**

Andersson et al.

**Serial No.:** To be assigned

**Filed:** 15 June 2001

**For:** SELECTING ANIMALS FOR  
PARENTALLY IMPRINTED TRAITS

**Examiner:** To be assigned

**Group Art Unit:** To be assigned

**Attorney Docket No.:** 4951US

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number: EL74037159US

Date of Deposit with USPS: 15 June 2001

Person making Deposit: Jared Turner

**PRELIMINARY AMENDMENT**

Commissioner for Patents  
Washington, D.C. 20231

Sir:

Please amend the above identified patent application as follows:

**IN THE CLAIMS:**

Claims 18-23 have been cancelled and new claims 28-38 have been added. Claims 1-17 and 24-27 have been amended herein. All cancellations of and amendments to claims are made without prejudice or disclaimer. All of the pending claims 1-17 and 24-38 are presented, pursuant to 37 C.F.R. §§ 1.121(c)(1)(i) and 1.121(c)(3), in clean form below. Please enter these claims as amended. Also attached is a marked-up version of the claims amended herein pursuant to 37 C.F.R. § 1.121(c)(1)(ii).

1. (Amended) A method for selecting an animal for having desired genotypic properties comprising testing said animal for the presence of a parentally imprinted quantitative trait locus (QTL).

2. (Amended) The method according to claim 1, further comprising testing a nucleic acid sample from said animal for the presence of a QTL.

3. (Amended) The method according to claim 1 wherein said animal comprises a pig and in said pig said QTL is located at chromosome 2.

4. (Amended) The method according to claim 1 wherein said animal comprises a pig and in said pig said QTL maps at about position 2p1.7.

5. (Amended) The method according to claim 1 wherein said QTL is related to the potential muscle mass and/or fat deposition of said animal.

6. (Amended) The method according to claim 5 wherein said QTL comprises at least a part of an insulin-like growth factor-2 gene.

7. (Amended) The method according to claim 1 wherein said animal comprises a pig and in said pig said QTL comprises a marker characterized as nt241 (G-A) or as Swc9.

8. (Amended) The method according to claim 1 wherein a paternal allele of said QTL is predominantly expressed in said animal.

9. (Amended) The method according to claim 1 wherein a maternal allele of said QTL is predominantly expressed in said animal.



10. (Amended) An isolated and/or recombinant nucleic acid comprising a parentally imprinted quantitative trait locus (QTL) or a functional fragment of said QTL comprising genetic information capable of influencing a quantitative trait of an animal.

11. (Amended) An isolated and/or recombinant nucleic acid comprising a synthetic parentally imprinted quantitative trait locus (QTL) derived from at least one chromosome or a functional fragment of said chromosome comprising genetic information capable of influencing a quantitative trait of an animal.

12. (Amended) The isolated and/or recombinant nucleic acid according to claim 10 at least partly derived from a *Sus scrofa* chromosome.

13. (Amended) The isolated and/or recombinant nucleic acid according to claim 12 wherein said nucleic acid is at least partly derived from a *Sus scrofa* chromosome 2.

14. (Amended) The nucleic acid according to claim 10 wherein said QTL is related to the potential muscle mass and/or fat deposition of said animal.

15. (Amended) The nucleic acid according to claim 10 wherein said QTL comprises at least a part of an insulin-like growth factor-2 gene.

16. (Amended) The nucleic acid according to claim 10 wherein a paternal allele of said QTL is capable of being predominantly expressed.

17. (Amended) The nucleic acid according to claim 10 wherein a maternal allele of said QTL is capable of being predominantly expressed.

24. (Amended) A transgenic animal comprising the isolated and/or recombinant nucleic acid according to claim 11.

25. (Amended) The transgenic animal according to claim 24 wherein said transgenic animal is a male.

26. (Amended) Sperm or an embryo derived from the transgenic animal according to claim 24.

27. (Amended) A method for breeding animals destined for slaughter comprising utilizing the sperm or embryo according to claim 26.

28. (New) The isolated and/or recombinant nucleic acid according to claim 13 wherein said isolated and/or recombinant nucleic acid is at least partly derived from a region mapping at about position 2p1.7.

29. (New) The isolated and/or recombinant nucleic acid according to claim 11 at least partly derived from a *Sus scrofa* chromosome.

30. (New) The isolated and/or recombinant nucleic acid according to claim 29 wherein said isolated and/or recombinant nucleic acid is at least partly derived from a *Sus scrofa* chromosome 2.

31. (New) The isolated and/or recombinant nucleic acid according to claim 30 wherein said isolated and/or recombinant nucleic acid is at least partly derived from a region mapping at about position 2p1.7.

32. (New) The isolated and/or recombinant nucleic acid according to claim 11 wherein said QTL is related to the potential muscle mass and/or fat deposition of said animal.

33. (New) The isolated and/or recombinant nucleic acid according to claim 11 wherein said QTL comprises at least a part of an inuslin-like growth factor-2 gene.

34. (New) The isolated and/or recombinant nucleic acid according to claim 11 wherein a paternal allele of said QTL is capable of being predominantly expressed.

35. (New) The isolated and/or recombinant nucleic acid according to claim 11 wherein a maternal allele of said QTL is capable of being predominantly expressed.

36. (New) The method according to claim 1 wherein said testing comprises utilizing an isolated and/or recombinant nucleic acid comprising a QTL or a functional fragment of said QTL comprising genetic information capable of influencing a quantitative trait of said animal.

37. (New) The method according to claim 36 wherein said animal comprises a breeding animal or an animal destined for slaughter and wherein said desired genotypic properties further comprise potential phenotypic properties.

38. (New) The method according to claim 37 wherein said desired genotypic properties are related to muscle mass and/or fat deposition.

## REMARKS

The application is to be amended without prejudice or disclaimer as previously set forth, which should not be viewed as narrowing or limiting the claims. The amendments are sought to conform the application to a form more consistent with Office practice by removing multiple dependencies. It is respectfully submitted that no new matter has been added by the amendments. Should the Office determine that additional issues remain, which might be resolved by a telephone conference, it is respectfully invited to contact applicants' undersigned attorney.

Respectfully Submitted,



Shawn G. Hansen  
Registration Number 42,627  
Attorney for Applicants  
TRASKBRITT, PC  
P.O. Box 2550  
Salt Lake City, Utah 84110  
Telephone: (801) 532-1922

Date: 15 June 2001

Enclosure: Version With Markings to Show Changes Made

N:\2183\4951\Preliminary Amendment.wpd

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS

1. (Amended) A method for selecting [a domestic] an animal for having desired genotypic properties comprising testing said animal for the presence of a parentally imprinted quantitative trait locus (QTL).

2. (Amended) [A] The method according to claim 1, further comprising testing a nucleic acid sample from said animal for the presence of a [parentally imprinted quantitative trait locus ([QTL])].

3. (Amended) [A] The method according to claim 1 [or 2 ]wherein said animal comprises a pig and in [the] said pig said QTL is located at chromosome 2.

4. (Amended) [A] The method according to claim [2 or 3] 1 wherein said animal comprises a pig and in said pig said QTL [is mapping] maps at [around] about position 2p1.7.

5. (Amended) [A] The method according to claim 1 [to 4] wherein said QTL is related to the potential muscle mass and/or fat deposition of said animal.

6. (Amended) [A] The method according to claim 5 wherein said QTL comprises at least a part of an insulin-like growth factor-2 [(IGF2)]gene.

7. (Amended) [A] The method according to [anyone of claims] claim 1 [to 6] wherein said animal comprises a pig and in [the] said pig said QTL comprises a marker characterized as nt241(G-A) or as Swc9[, as identified in figure 4].

8. (Amended) [A] The method according to [anyone of claims 1-7] claim 1 wherein a paternal allele of said QTL is predominantly expressed in said animal.

9. (Amended) [A] The method according to [anyone of claims 1-7] claim 1 wherein a maternal allele of said QTL is predominantly expressed in said animal.

10. (Amended) An isolated and/or recombinant nucleic acid comprising a parentally imprinted quantitative trait locus (QTL) or a functional fragment [derived thereof] of said QTL comprising genetic information capable of influencing a quantitative trait of an animal.

11. (Amended) An isolated and/or recombinant nucleic acid comprising a synthetic parentally imprinted quantitative trait locus (QTL) derived from at least one chromosome or a functional fragment [derived thereof.] of said chromosome comprising genetic information capable of influencing a quantitative trait of an animal.

12. (Amended) [A] The isolated and/or recombinant nucleic acid according to claim 10 [or 11] at least partly derived from a Sus scrofa chromosome.

13. (Amended) [A] The isolated and/or recombinant nucleic acid according to claim 12 wherein said nucleic acid is at least partly derived from a Sus scrofa chromosome 2[, preferably from a region mapping at around position 2p1.7].

14. (Amended) [A] The nucleic acid according to [any one of claims] claim 10 [to 13] wherein said QTL is related to the potential muscle mass and/or fat deposition of said animal.

15. (Amended) [A] The nucleic acid according to [any one of claims] claim 10 [to 14] wherein said QTL comprises at least a part of [a] an insulin-like growth factor-2 [(IGF2) ]gene.

16. (Amended) [A] The nucleic acid according to [anyone of claims] claim 10 [to 15] wherein a paternal allele of said QTL is capable of being predominantly expressed.

17. (Amended) [A] The nucleic acid according to [anyone of claims] claim 10 [to 16] wherein a maternal allele of said QTL is capable of being predominantly expressed.

24. (Amended) A transgenic animal comprising the isolated and/or recombinant [a] nucleic acid according to claim 11 [anyone of claims 11 to 16].

25. (Amended) [An animal]The transgenic animal according to [anyone of claims 21-24 which]claim 24 wherein said transgenic animal is a male.

26. (Amended) Sperm or an embryo derived from [an]the transgenic animal according to [anyone of claims 21-25]claim 24.

27. (Amended) [Use of a sperm or an embryo according to claim 26 in] A method for breeding animals destined for slaughter comprising utilizing the sperm or embryo according to claim 26.



**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**In re Application of:**

**ANDERSSON et al.**

**Serial No.: 09/868,732**

**Filed: June 15, 2001**

**For: SELECTING ANIMALS FOR  
PARENTALLY IMPRINTED TRAITS**

**Examiner: To be assigned**

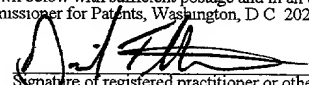
**Group Art Unit: To be assigned**

**Attorney Docket No.: 4951US**

**CERTIFICATE OF MAILING**

I hereby certify that this correspondence along with any attachments referred to or identified as being attached or enclosed is being deposited with the United States Postal Service as First Class Mail (under 37 C.F.R. § 1.8(a)) on the date of deposit shown below with sufficient postage and in an envelope addressed to the Commissioner for Patents, Washington, D.C. 20231

9/17/01  
Date of Deposit

  
Signature of registered practitioner or other person  
having reasonable basis to expect mailing to occur on  
date of deposit shown pursuant to 37 C.F.R. §  
1.8(a)(1)(ii)

Daniel Thatcher  
Typed/printed name of person whose signature is  
contained above

**AMENDMENT**

Box Non-Fee Amendment  
Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination of the above-referenced patent application on the merits, entry of the amendments as set forth herein is respectfully solicited.



IN THE SPECIFICATION:

Pursuant to 37 C.F.R. §§ 1.121 and 1.125 (as amended to date) please enter the substitute specification in clean form and including paragraph numbers [0001] through [0085] attached hereto as Appendix A. A marked-up substitute specification to clearly identify amendments to the specification as required by 37 C.F.R. § 1.121(b)(3)(iii) is attached as Appendix B. It is respectfully submitted that the substitute specification does not introduce new matter into the above-referenced patent application.

**REMARKS**

No new matter has been added. The Applicants again request entry of the amendments as set forth in the Appendices hereto prior to examination of the application on the merits.

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'Allen C. Turner', with a long horizontal flourish extending to the right.

Allen C. Turner

Registration No. 33,041

Attorney for Applicants

TRASKBRITT, PC

P. O. Box 2550

Salt Lake City, Utah 84110-2550

Telephone: (801) 532-1922

Date: September 17, 2001

ACT/dn

N:\2183\4951\Amendment - 091501

# **APPENDIX A**

**(CLEAN VERSION OF SUBSTITUTE SPECIFICATION EXCLUDING CLAIMS)**

**(Serial No. 09/868,732)**

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number: \_\_\_\_\_

Date of Deposit with USPS: \_\_\_\_\_

Person making Deposit: \_\_\_\_\_

APPLICATION FOR LETTERS PATENT

for

**SELECTING ANIMALS FOR PARENTALLY IMPRINTED TRAITS**

Inventor:

Andersson, Leif  
Georges, Michel  
Spincemaille, Geert

Attorney:  
Allen C. Turner  
Registration No. 33,041  
TRASKBRITT  
P.O. Box 2550  
Salt Lake City, Utah 84110  
(801) 532-1922

## SELECTING ANIMALS FOR PARENTALLY IMPRINTED TRAITS

### TECHNICAL FIELD

**[0001]** The invention relates to methods to select breeding animals or animals destined for slaughter for having desired genotypic or potential phenotypic properties, in particular related to muscle mass and/or fat deposition.

### BACKGROUND OF THE INVENTION

**[0002]** Breeding schemes for domestic animals have so far focused on farm performance traits and carcass quality. This has resulted in substantial improvements in traits like reproductive success, milk production, lean/fat ratio, prolificacy, growth rate and feed efficiency. Relatively simple performance test data have been the basis for these improvements, and selected traits were assumed to be influenced by a large number of genes, each of small effect (the infinitesimal gene model). There are now some important changes occurring in this area. First, the breeding goal of some breeding organisations has begun to include meat quality attributes in addition to the “traditional” production traits. Second, evidence is accumulating that current and new breeding goal traits may involve relatively large effects (known as major genes), as opposed to the infinitesimal model that has been relied on so far.

**[0003]** Modern DNA-technologies provide the opportunity to exploit these major genes, and this approach is a very promising route for the improvement of meat quality, especially since direct meat quality assessment is not viable for potential breeding animals. Also for other traits such as lean/fat ratio, growth rate and feed efficiency, modern DNA technology can be very effective. Also these traits are not always easy to measure in the living animal.

**[0004]** The evidence for several of the major genes was originally obtained using segregation analysis, i.e., without any DNA marker information. Afterwards, molecular studies were performed to detect the location of these genes on the genetic map. In practice, and except for alleles of very large effect, DNA studies are required to dissect the genetic nature of most traits of economic importance. DNA markers can be used to localise genes or alleles responsible for qualitative traits like coat color, and they can also be used to detect genes or alleles with substantial effects on

quantitative traits like growth rate, IMF, etc. In this case, the approach is referred to as QTL (quantitative trait locus) mapping, wherein a QTL comprises at least a part of the nucleic acid genome of an animal where genetic information capable of influencing said quantitative trait (in said animal or in its offspring) is located. Information at the DNA level can not only help to fix a specific major gene in a population, but also assist in the selection of a quantitative trait which is already selected for. Molecular information in addition to phenotypic data can increase the accuracy of selection and therefore the selection response.

**[0005]** Improving meat quality or carcass quality is not just about changing levels of traits like tenderness or marbling, but it is also about increasing uniformity. The existence of major genes provides excellent opportunities for improving meat quality because it allows large steps to be made in the desired direction. It will help to reduce variation, since we can fix relevant genes in our products. Another aspect is that selecting for major genes allows differentiation for specific markets. Studies are underway in several species, particularly, pigs, sheep, deer and beef cattle.

**[0006]** In particular, intense selection for meat production has resulted in animals with extreme muscularity and leanness in several livestock species. In recent years it has become feasible to map and clone several of the genes causing these phenotypes, paving the way towards more efficient marker-assisted selection, targeted drug development (performance-enhancing products) and transgenesis. Mutations in the ryanodine receptor (Fuji et al, 1991; MacLennan and Phillips, 1993) and myostatin (Grobet et al, 1997; Kambadur et al, 1997; McPherron and Lee, 1997) have been shown to cause muscular hypertrophies in pigs and cattle respectively, while genes with major effects on muscularity and/or fat deposition have, for instance, been mapped to pig chromosome 4 (Andersson et al, 1994) and sheep chromosome 18 (Cocket et al, 1996).

## DISCLOSURE OF THE INVENTION

**[0007]** However, although there have been successes in identifying QTLs, the information is currently of limited use within commercial breeding programmes. Many workers in this field conclude that it is necessary to identify the particular genes underlying the QTL. This is a substantial task, as the QTL region is usually relatively large and may contain many genes. Identification of the relevant genes from the many that may be involved thus remains a significant hurdle in farm animals.

[0008] The invention provides a method for selecting a domestic animal for having desired genotypic or potential phenotypic properties comprising testing the animal for the presence of a parentally imprinted qualitative or quantitative trait locus (QTL). Herein, a domestic animal is defined as an animal being selected or having been derived from an animal having been selected for having desired genotypic or potential phenotypic properties.

[0009] Domestic animals provide a rich resource of genetic and phenotypic variation; traditionally, domestication involves selecting an animal or its offspring for having desired genotypic or potential phenotypic properties. This selection process has in the past century been facilitated by growing understanding and utilisation of the laws of Mendelian inheritance. One of the major problems in breeding programmes of domestic animals is the negative genetic correlation between reproductive capacity and production traits. This is, for example, the case in cattle (a high milk production generally results in slim cows and bulls), poultry (broiler lines have a low level of egg production and layers have generally very low muscle growth), pigs (very prolific sows are, in general, fat and have comparatively less meat) or sheep (high prolific breeds have low carcass quality and vice versa). The invention now provides that knowledge of the parental imprinting character of various traits allows to select, for example, sire lines homozygous for a paternally imprinted QTL, for example, linked with muscle production or growth; the selection for such traits can thus be less stringent in dam lines in favour of the reproductive quality. The phenomenon of genetic or parental imprinting has never been utilised in selecting domestic animals; it was never considered feasible to employ this elusive genetic characteristic in practical breeding programmes. The invention provides a breeding programme, wherein knowledge of the parental imprinting character of a desired trait, as demonstrated herein, results in a breeding programme, for example, a BLUP programme, with a modified animal model. This increases the accuracy of the breeding value estimation and speeds up selection compared to conventional breeding programmes. Until now, the effect of a parentally imprinted trait in the estimation of a conventional BLUP programme was neglected; using and understanding the parental character of the desired trait, as provided by the invention, allows selecting on parental imprinting, even without DNA testing. For example, selecting genes characterised by paternal imprinting is provided to help increase uniformity; a (terminal) parent homozygous for the “good or wanted” alleles will pass them to all offspring, regardless of the other parent’s alleles, and

the offspring will all express the desired parent's alleles. This results in more uniform offspring. Alleles that are interesting or favourable from the maternal side are often the ones that have opposite effects to alleles from the paternal side. For example, in meat animals such as pigs, alleles linked with meat quality traits such as intramuscular fat or muscle mass could be fixed in the dam lines while alleles linked with reduced backfat could be fixed in the sire lines. Other desirable combinations are, for example, fertility and/or milk yield in the female line with growth rates and/or muscle mass in the male lines.

**[0010]** In a preferred embodiment, the invention provides a method for selecting a domestic animal for having desired genotypic or potential phenotypic properties comprising testing a nucleic acid sample from the animal for the presence of a parentally imprinted quantitative trait locus (QTL). A nucleic acid sample can, in general, be obtained from various parts of the animal's body by methods known in the art. Traditional samples for the purpose of nucleic acid testing are blood samples or skin or mucosal surface samples, but samples from other tissues can be used as well; in particular, sperm samples, oocyte or embryo samples can be used. In such a sample, the presence and/or sequence of a specific nucleic acid, be it DNA or RNA, can be determined with methods known in the art, such as hybridisation or nucleic acid amplification or sequencing techniques known in the art. The invention provides testing such a sample for the presence of nucleic acid wherein a QTL or allele associated therewith is associated with the phenomenon of parental imprinting, for example, where it is determined whether a paternal or maternal allele of said QTL is capable of being predominantly expressed in the animal.

**[0011]** The purpose of breeding programmes in livestock is to enhance the performances of animals by improving their genetic composition. In essence, this improvement accrues by increasing the frequency of the most favourable alleles for the genes influencing the performance characteristics of interest. These genes are referred to as QTL. Until the beginning of the nineties, genetic improvement was achieved via the use of biometrical methods, but without molecular knowledge of the underlying QTL.

**[0012]** Since the beginning of the nineties and due to recent developments in genomics, it is conceivable to identify the QTL underlying a trait of interest. The invention now provides identifying and using parentally imprinted QTLs which are useful for selecting animals by mapping quantitative



trait loci. Again, the phenomenon of genetic or paternal imprinting has never been utilised in selecting domestic animals; it was never considered feasible to employ this elusive genetic characteristic in practical breeding programmes. For example, Kovacs and Kloting (Biochem. Mol. Biol. Int. 44:399-405, 1998), where parental imprinting is not mentioned, and not suggested, found linkage of a trait in female rats, but not in males, suggesting a possible sex specificity associated with a chromosomal region, which, of course, excludes parental imprinting, a phenomenon wherein the imprinted trait of one parent is preferably but genderaspecifically expressed in his or her offspring.

## BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

### Legends to the Figures

**[0013]** Fig. 1: Test statistic curves obtained in QTL analyses of chromosome 2 in a Wild Boar/Large White intercross. The graph plots the F ratio testing the hypothesis of a single QTL at a given position along the chromosome for the traits indicated. The marker map with the distances between markers in Kosambi centiMorgan is given on the X-axis. The horizontal lines represent genome-wise significant ( $P < 0.05$ ) and suggestive levels for the trait lean meat in ham; similar significance thresholds were obtained for the other traits.

**[0014]** Figure 2: Piétrain pig with characteristic muscular hypertrophy.

**[0015]** Figures 3A-3C: Lodscore curves obtained in a Piétrain x Large White intercross for six phenotypes measuring muscle mass and fat deposition on pig chromosome 2. The most likely positions of the *IGF2* and *MyoD* genes determined by linkage analysis with respect to the microsatellite marker map are shown.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  as testing for the presence of a Mendelian QTL,  $H_2$  as testing for the presence of a paternally expressed QTL, and  $H_3$  as testing for the presence of a maternally expressed QTL. 3A:  $\log_{10}(H_1/H_0)$ , 3B:  $\log_{10}(H_2/H_0)$ , 3C:  $\log_{10}(H_3/H_0)$ .

**[0016]** Figure 4: A. Structure of the human *IGF2* gene according to ref. 17, with aligned porcine adult liver cDNA sequence as reported in ref. 16. The position of the *nt241(G-A)* transition and *Swc9* microsatellite are shown. B. The corresponding markers were used to demonstrate the

monoallelic (paternal) expression of IGF2 in skeletal muscle and liver of 10-week old fetuses. PCR amplification of the *nt421* (G-A) polymorphism and *Swc9* microsatellite from genomic DNA clearly shows the heterozygosity of the fetus, while only the paternal allele is detected in liver cDNA (*nt421*(G-A) and *Swc9*) and muscle cDNA (*Swc9*). The absence of RT-PCR product for *nt421*(G-A) in fetal muscle points towards the absence of mRNA including exon 2 in this tissue. Parental origin of the fetal alleles was determined from the genotypes of the sire and dam (data not shown).

[0017] Figure 5: A NotI restriction map showing the relative position of BAC-PIGF2-1 (comprising INS and IGF2 genes), and BAC-PIGF2-2 (comprising IGF2 and H19 genes).

[0018] Figure 6: Nucleic acid sequences of contig 1 to contig 115 derived from BAC-PIGF2-1 which was shotgun sequenced using standard procedures and automatic sequencers.

[0019] Figure 7: Similarity between porcine contigs of figure 6 and orthologous sequences in human.

[0020] Figure 8: Nucleic acid sequences of contig 1 to contig 7 derived from BAC-PIGF2-2, (the 24 Kb NotI fragment not present in BAC-PIGF2-1) which was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers.

[0021] Figure 9: Similarity between porcine contigs of figure 8 and orthologous sequences in human.

[0022] Figure 10: DNA sequence polymorphisms in the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals.

## DETAILED DESCRIPTION OF THE INVENTION

[0023] The invention provides the initial localisation of a parentally imprinted QTL on the genome by linkage analysis with genetic markers, and the actual identification of the parentally imprinted gene(s) and causal mutations therein. Molecular knowledge of such a parentally imprinted QTL allows for more efficient breeding designs herewith provided. Applications of molecular knowledge of parentally imprinted QTLs in breeding programmes include: marker-assisted segregation analysis to identify the segregation of functionally distinct, parentally imprinted QTL alleles in the populations of interest, marker-assisted selection (MAS) performed within lines to enhance genetic response by increasing selection accuracy or selection intensity or by reducing the

generation interval using the understanding of the phenomenon of parental imprinting, marker-assisted introgression (MAI) to efficiently transfer favourable parentally imprinted QTL alleles from a donor to a recipient population, genetic engineering of the identified parentally imprinted QTL and genetic modification of the breeding stock using transgenic technology, and development of performance enhancing products using targeted drug development exploiting molecular knowledge of said QTL.

**[0024]** The inventors undertook two independent experiments to determine the practical use of parental imprinting of a QTL.

**[0025]** In a first experiment, performed in a previously described Piétrain x Large White intercross, the likelihood of the data were computed under a model of paternal (paternal allele only expressed) and maternal imprinting (maternal allele only expressed) and compared with the likelihood of the data under a model of a conventional "Mendelian" QTL. The results strikingly demonstrated that the QTL was indeed paternally expressed, the QTL allele (Piétrain or Large White) inherited from the F<sub>1</sub> sow having no effect whatsoever on the carcass quality and quantity of the F<sub>2</sub> offspring. It was seen that very significant lodscores were obtained when testing for the presence of a paternally expressed QTL, while there was no evidence at all for the segregation of a QTL when studying the chromosomes transmitted by the sows. The same tendency was observed for all traits, showing that the same imprinted gene is responsible for the effects observed on the different traits. Table 1 reports the maximum likelihood (ML) phenotypic means for the F<sub>2</sub> offspring sorted by inherited paternal QTL allele.

**[0026]** In a second experiment performed in the Wild Boar X Large White intercross, QTL analyses of body composition, fatness, meat quality, and growth traits were carried out with the chromosome 2 map using a statistical model testing for the presence of an imprinting effect. Clear evidence for a paternally expressed QTL located at the very distal tip of 2p was obtained (Fig. 2; Table I). The clear paternal expression of a QTL is illustrated by the least squares means which fall into two classes following the population origin of the paternally inherited allele (Table 1). For a given paternally imprinted QTL, implementation of marker-assisted segregation analysis, selection (MAS) and introgression (MAI) can be performed using genetic markers that are linked to the QTL,

using genetic markers that are in linkage disequilibrium with the QTL, or using the actual causal mutations within the QTL.

**[0027]** Understanding the parent-of-origin effect characterising a QTL allows for its optimal use in breeding programmes. Indeed, marker-assisted segregation analysis under a model of parental imprinting will yield better estimates of QTL allele effects. Moreover, it allows for the application of specific breeding schemes to optimally exploit a QTL. In one embodiment of the invention, the most favourable QTL alleles would be fixed in breeding animal lines and, for example, used to generate commercial, crossbred males by marker-assisted selection (MAS, within lines) and marker-assisted introgression (MAI, between lines). In another embodiment, the worst QTL alleles would be fixed in the animal lines used to generate commercial crossbred females by MAS (within lines) and MAI (between lines).

**[0028]** In a preferred embodiment of the invention, the animal is a pig. Note, for example, that the invention provides the insight that today half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit an unfavourable Large White muscle mass QTL as provided by the invention, causing considerable loss, and the invention now, for example, provides the possibility to select the better half of the population in that respect. However, it is also possible to select commercial sow lines enriched within the boars unfavourable alleles, allowing the sows to be equipped with other alleles more desirable for, for example, reproductive purposes.

**[0029]** In a preferred embodiment of a method provided by the invention, said QTL is located at a position corresponding to a QTL located at chromosome 2 in the pig. For example, it is known to form comparative mapping data between pig and human, including bidirectional chromosome painting and that SSC2p is homologous to HSA11pter-q13<sup>11, 12</sup>. HSA11pter-q13 is known to harbour a cluster of imprinted genes: IGF2, INS2, H19, MAH2, P57<sup>KIP2</sup>, K<sub>v</sub>LQTL1, Tapa1./CD81, Orct12, Impt1 and Ip1. The cluster of imprinted genes located in HSA11pter-q13 is characterised by 8 maternally expressed genes: H19, MASH2, P57<sup>KIP2</sup>, K<sub>v</sub>LQTL1, TAPA1/CD81, ORCTL2, IMPT1 and IPI, and two paternally expressed genes: IGF2 and INS. However, Johanson et al. (Genomics 25:682-690, 1995) and Reik et al. (Trends in Genetics, 13:330-334, 1997) show that the whereabouts of these loci in various animals are not clear. For example, the HSA11 and MMU7 loci do not correspond among each other, the MMU7 and the SSC2 loci do not correspond, whereas the HSA11

and SSC2 loci seem to correspond, and no guidance is given where one or more of, for example, the above-identified parentally expressed individual genes are localised on the three species' chromosomes.

**[0030]** Other domestic animals, such as cattle, sheep, poultry and fish, having similar regions in their genome harbouring such a cluster of imprinted genes or QTLs, the invention herewith provides use of these orthologous regions of other domestic animals in applying the phenomenon of parental imprinting in breeding programmes. In pigs, said cluster is mapped at around position 2p1.7 of chromosome 2; however, a method as provided by the invention employing (fragments of) said maternally or paternally expressed orthologous or homologous genes or QTLs is advantageously used in other animals as well as for breeding and selecting purposes. For example, a method is provided wherein said QTL is related to the potential muscle mass and/or fat deposition, preferably with limited effects on other traits such as meat quality and daily gain of the animal or wherein said QTL comprises at least a part of an insulin-like growth factor-2 (IGF2) allele. Reik et al. (Trends in Genetics, 13:330-334, 1997) explain that this gene in humans is related to Beckwith-Wiedemann syndrome, an apparently parentally imprinted disease syndrome most commonly seen with human fetuses, where the gene has an important role in prenatal development. No relationship is shown or suggested with postnatal development relating to muscle development or fatness in (domestic) animals.

**[0031]** In a preferred embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7. In particular, the invention relates to the use of genetic markers for the telomeric end of pig chromosome 2p in marker selection (MAS) of a parentally imprinted Quantitative Trait Locus (QTL) affecting carcass yield and quality in pigs. Furthermore, the invention relates to the use of genetic markers associated with the IGF2 locus in MAS in pigs, such as polymorphisms and microsatellites and other characterising nucleic acid sequences shown herein, such as shown in figures 4 to 10. In a preferred embodiment, the invention provides a QTL located at the distal tip of *Sus scrofa* chromosomes 2 with effects on various measurements of carcass quality and quantity, particularly muscle mass and fat deposition.

[0032] In a first experiment, a QTL mapping analysis was performed in a Wild Boar X Large White intercross counting 200 F<sub>2</sub> individuals. The F<sub>2</sub> animals were sacrificed at a live weight of at least 80 kg or at a maximum age of 190 days. Phenotypic data on birth weight, growth, fat deposition, body composition, weight of internal organs, and meat quality were collected; a detailed description of the phenotypic traits are provided by Andersson et al.<sup>1</sup> and Andersson-Eklund et al.<sup>4</sup>

[0033] A QTL (without any significant effect on back-fat thickness) at an unspecified locus on the proximal end of chromosome 2 with moderate effect on muscle mass, and located about 30cM away from the parentally imprinted QTL reported here, was previously reported by the inventors; whereas the QTL as now provided has a very large effect, explaining at least 20-30% of variance, making the QTL of the present invention commercially very attractive, which is even more so because the present QTL is parentally imprinted. The marker map of chromosome 2p was improved as part of this invention by adding microsatellite markers in order to cover the entire chromosome arm. The following microsatellite markers were used: *Swc9*, *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. QTL analyses of body composition, fatness, meat quality, and growth traits were carried out with the new chromosome 2 map. Clear evidence for a QTL located at the very distal tip of 2p was obtained (Fig. 1; Table 1). The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F<sub>2</sub> population. Large effects on the area of the longissimus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population.

[0034] In a second experiment, QTL mapping was performed in a Piétrain X Large White intercross comprising 1125 F<sub>2</sub> offspring. The Large White and Piétrain parental breeds differ for a number of economically important phenotypes. Piétrains are famous for their exceptional muscularity and leanness<sup>10</sup> (Figure 2), while Large Whites show superior growth performance. Twenty-one distinct phenotypes measuring growth performance (5), muscularity (6), fat deposition (6), and meat quality (4), were recorded on all F<sub>2</sub> offspring. In order to map QTL underlying the genetic differences

between these breeds, the inventors undertook a whole genome scan using microsatellite markers on an initial sample of 677 F<sub>2</sub> individuals. The following microsatellite marker map was used to analyse chromosome 2; SW2443, SWC9 and SW2623, SWR2516- (0,20) -SWR783- (0,29) -SW240- (0,20) -SW776- (0,08) -S0010- (0, 04) -SW1695-(0,36) -SWR308. Analysis of pig chromosome 2 using a Maximum Likelihood multipoint algorithm revealed highly significant lodscores (up to 20) for three of the six phenotypes measuring muscularity (% lean cuts, % ham, % loin) and three of the six phenotypes measuring fat deposition (back-fat thickness (BFT), % backfat, % fat cuts) at the distal end of the short arm of chromosome 2 (Figure 1). Positive lodscores were obtained in the corresponding chromosome region for the remaining six muscularity and fatness phenotypes, however, not reaching the experiment-wise significance threshold ( $\alpha=5\%$ ). There was no evidence for an effect of the corresponding QTL on growth performance (including birth weight) or recorded meat quality measurements (data not shown). To confirm this finding, the remaining sample of 355 F<sub>2</sub> offspring was genotyped for the four most distal 2p markers and QTL analysis performed for the traits yielding the highest lodscores in the first analysis. Lodscores ranged from 2.1 to 7.7, clearly confirming the presence of a major QTL in this region. Table 2 reports the corresponding ML estimates for the three genotypic means as well as the residual variance. Evidence based on marker-assisted segregation analysis points towards residual segregation at this locus within the Piétrain population.

**[0035]** These experiments, therefore, clearly indicated the existence of a QTL with a major effect on carcass quality and quantity on the telomeric end of pig chromosome arm 2p, the likely existence of an allelic series at this QTL with at least three alleles: Wild-Boar < Large White < Piétrain, and possibly more given the observed segregation within the Piétrain breed.

**[0036]** The effects of the identified QTL on muscle mass and fat deposition are truly major, being of the same magnitude of those reported for the *CRC* locus though apparently without the associated deleterious effects on meat quality. We estimate that both loci jointly explain close to 50% of the Piétrain versus Large White breed difference for muscularity and leanness. The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F<sub>2</sub> population. Large effects on the area of the longissimus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect

an one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL, when compared to the Wild Boar allele, was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population. The strong imprinting effect observed for all affected traits shows that a single causative locus is involved. The pleiotropic effects on skeletal muscle mass and the size of the heart appear adaptive from a physiological point of view as a larger muscle mass requires a larger cardiac output.

**[0037]** In a further embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7., wherein said QTL comprises at least a part of a *Sus scrofa* insulin-like growth factor-2 (IGF2) allele or a genomic area closely related thereto, such as polymorphisms and microsatellites and other characterising nucleic acid sequences shown herein, such as shown in figures 4 to 10. The important role of IGF2 for prenatal development is well-documented from knockout mice as well as from its causative role in the human Beckwith-Wiedemann syndrome. This invention demonstrates an important role for the IGF2-region, also for postnatal development.

**[0038]** To show the role of IGF2, the inventors performed the following three experiments:

**[0039]** A genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong consistent signal on the terminal part of chromosome 2p.

**[0040]** A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IGF2 3'UTR using the BAC clone. A complex microsatellite was identified about 800bp downstream of the stop codon; a sequence comparison revealed that this microsatellite was identical to a previously described anonymous microsatellite, *Swc9*<sup>6</sup>. This marker was used in the initial QTL mapping experiments and its location on the genetic map correspond with the most likely position of the QTL both in the Piétrain X Large White and in the Large White x Wild Boar pedigree.



**[0041]** Analysis of skeletal muscle and liver cDNA from 10-week old fetuses heterozygous for a nt241 (G-A) transversion in the second exon of the porcine IGF2 gene and SWC9 shows that the IGF2 gene is imprinted in these tissues in the pig as well and only expressed from the paternal allele.

**[0042]** Based on a published porcine adult liver cDNA sequence<sup>16</sup>, the inventors designed primer pairs allowing amplification of the entire IGF2 coding sequence with 222 bp of leader and 280 bp of trailer sequence from adult skeletal muscle cDNA. Piétrain and Large White RT-PCR products were sequenced, indicating that the coding sequences are identical in both breeds and with the published sequence. However, a G→A transition was found in the leader sequence corresponding to exon 2 in man. Following conventional nomenclature, this polymorphism will be referred to as *nt241* (G-A). We developed a screening test for this single nucleotide polymorphism (SNP) based on the ligation amplification reaction (LAR), allowing us to genotype our pedigree material. Based on these data, IGF2 was shown to colocalize with the SWC9 microsatellite marker ( $\theta=0\%$ ), therefore virtually coinciding with the most likely position of the QTL, and well within the 95% support interval for the QTL. Subsequent sequence analysis demonstrated that the microsatellite marker SWC9 is actually located within the 3'UTR of the IGF2 gene.

**[0043]** As previously mentioned, the knowledge of this QTL provides a method for the selection of animals such as pigs with improved carcass merit. Different embodiments of the invention are envisaged, including: marker-assisted segregation analysis to identify the segregation of functionally distinct QTL alleles in the populations of interest; marker-assisted selection (MAS) performed within lines to enhance genetic response by increasing selection accuracy or selection intensity or by reducing the generation interval; and marker-assisted introgression (MAI) to efficiently transfer favourable QTL alleles from a donor to a recipient population, thereby enhancing genetic response in the recipient population. Implementation of embodiments of marker-assisted segregation analysis, selection (MAS) and introgression (MAI) can be performed using genetic markers that are linked to the QTL, genetic markers that are in linkage disequilibrium with the QTL, and the actual causal mutations within the QTL.

[0044] In a further embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a Sus scrofa chromosome 2 mapping at position 2p1.7., wherein said QTL is paternally expressed, i.e., is expressed from the paternal allele. In man and mouse, IGF2 is known to be imprinted and to be expressed exclusively from the paternal allele in several tissues. Analysis of skeletal muscle cDNA from pigs heterozygous for the SNP and/or SWC9 shows that the same imprinting holds in the pig as well. Understanding the parent-of-origin effect characterising the QTL as provided by the invention now allows for its optimal use in breeding programmes. Indeed, today, half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit the unfavourable Large White allele, causing considerable loss. Using a method as provided by the invention avoids this problem.

[0045] The invention furthermore provides an isolated and/or recombinant nucleic acid or functional fragment derived thereof comprising a parentally imprinted quantitative trait locus (QTL) or fragment thereof capable of being predominantly expressed by one parental allele. Having such a nucleic acid as provided by the invention available allows constructing transgenic animals wherein favourable genes are capable of being exclusively or predominantly expressed by one parental allele, thereby equipping the offspring of the animal homozygous for a desired trait with desired properties related to that parental allele that is expressed.

[0046] In a preferred embodiment, the invention provides an isolated and/or recombinant nucleic acid or fragment derived thereof comprising a synthetic parentally imprinted quantitative trait locus (QTL) or functional fragment thereof derived from at least one chromosome. Synthetic herein describes a parentally expressed QTL wherein various elements are combined that originate from distinct locations from the genome of one or more animals. The invention provides recombinant nucleic acid wherein sequences related to parental imprinting of one QTL are combined with sequences relating to genes or favourable alleles of a second QTL. Such a gene construct is favourably used to obtain transgenic animals wherein the second QTL has been equipped with paternal imprinting, as opposed to the inheritance pattern in the native animal from which the second QTL is derived. Such a second QTL can, for example, be derived from the same chromosome where the parental imprinting region is located, but can also be derived from a different chromosome from

the same or even a different species. In the pig, such a second QTL can, for example, be related to an oestrogen receptor (ESR)-gene (Rothschild et al., PNAS, 93, 201-201, 1996) or a FAT-QTL (Andersson, Science, 263, 1771-1774, 1994) for example, derived from another pig chromosome, such as chromosome 4. A second or further QTL can also be derived from another (domestic) animal or a human.

**[0047]** The invention furthermore provides an isolated and/or recombinant nucleic acid or functional fragment derived thereof at least partly corresponding to a QTL of a pig located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7 wherein said QTL is related to the potential muscle mass and/or fat deposition of said pig and/or wherein said QTL comprises at least a part of a *Sus scrofa* insulin-like growth factor-2 (IGF2) allele, preferably at least spanning a region between INS and H19, or preferably derived from a domestic pig, such as a Piétrain, Meishan, Duroc, Landrace or Large White, or from a Wild Boar. For example, a genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAG clone gave a strong, consistent signal on the terminal part of chromosome 2p. A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IGF2 3'UTR using the BAC clone. A complex microsatellite was identified about 800 bp downstream of the stop codon; a sequence comparison revealed that this microsatellite is identical to a previously described anonymous microsatellite, *Swc9*. PGR primers were designed and the microsatellite (IGF2ms) was found to be highly polymorphic with three different alleles among the two Wild Boar founders and another two among the eight Large White founders. IGF2ms was fully informative in the intercross as the breed of origin as well as the parent of origin could be determined with confidence for each allele in each F<sub>2</sub> animal.

**[0048]** A linkage analysis using the intercross pedigree was carried out with IGF2ms and the microsatellites *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. IGF2 was firmly assigned to 2p by highly significant lodscores (e.g. Z=89.0,  $\theta$ = 0.003 against *Swr2516*). Multipoint analyses, including previously typed chromosome 2 markers, revealed the following order of loci (sex-average map distances in Kosambi cM): *Sw2443*/*Swr2516*-0.3-IGF2-14.9-*Sw2623*-10.3-*Sw256*. No recombinant was observed between *Sw2443* and *Swr2516*, and the suggested proximal location

of IGF2 in relation to these loci is based on a single recombinant giving a lodscore support of 0.8 for the reported order. The most distal marker in our previous QTL study, *Sw256*, is located about 25 cM from the distal end of the linkage group.

**[0049]** The invention furthermore provides use of a nucleic acid or functional fragment derived thereof according to a method according to the invention. In a preferred embodiment, use of a method according to the invention is provided to select a breeding animal or animal destined for slaughter, or embryos or semen derived from these animals, for having desired genotypic or potential phenotypic properties. In particular, the invention provides such use wherein said properties are related to muscle mass and/or fat deposition. The QTL as provided by the invention may be exploited or used to improve, for example, lean meat content or back-fat thickness by marker-assisted selection within populations or by marker-assisted introgression of favourable alleles from one population to another. Examples of marker-assisted selection using the QTL as provided by the invention are use of marker-assisted segregation analysis with linked markers or with markers in disequilibrium to identify functionally distinct QTL alleles. Furthermore, identification of a causative mutation in the QTL is now possible, again leading to identify functionally distinct QTL alleles. Such functionally distinct QTL alleles located at the distal tip of chromosome 2p with large effects on skeletal muscle mass, the size of the heart, and on back-fat thickness are also provided by the invention. The observation of a similar QTL effect in a Large White x Wild Boar as well as in a Piétrain x Large White intercross provides proof of the existence of a series of at least three distinct functional alleles. Moreover, preliminary evidence based on marker-assisted segregation analysis points towards residual segregation at this locus within the Piétrain population (data not shown). The occurrence of an allelic series as provided by the invention allows identifying causal polymorphisms which - based on the quantitative nature of the observed effect - are unlikely to be gross gene alterations but rather subtle regulatory mutations. The effects on muscle mass of the three alleles rank in the same order as the breeds in which they are found, i.e., Piétrain pigs are more muscular than Large White pigs that in turn have higher lean meat content than Wild Boars. The invention furthermore provides use of the alleles as provided by the invention within line selection or for marker-assisted introgression using linked markers, markers in disequilibrium or alleles comprising causative mutations.

[0050] The invention furthermore provides an animal selected by using a method according to the invention. For example, a pig characterised in being homozygous for an allele in a QTL located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7 can now be selected and is thus provided by the invention. Since said QTL is related to the potential muscle mass and/or fat deposition of said pig and/or said QTL comprises at least a part of a *Sus scrofa* insulin-like growth factor-2 (IGF2) allele, it is possible to select promising pigs to be used for breeding or to be slaughtered. In particular, an animal according to the invention which is a male is provided. Such a male, or its sperm or an embryo derived thereof, can advantageously be used in breeding animals for creating breeding lines or for finally breeding animals destined for slaughter. In a preferred embodiment of such use as provided by the invention, a male, or its sperm, deliberately selected for being homozygous for an allele causing the extreme muscular hypertrophy and leanness, is used to produce offspring heterozygous for such an allele. Due to said allele's paternal expression, said offspring will also show the favourable traits, for example, related to muscle mass, even if the parent female has a different genetic background. Moreover, it is now possible to positively select the female(s) for having different traits, for example, related to fertility, without having a negative effect on the muscle mass trait that is inherited from the allele from the selected male. For example, earlier such males could occasionally be seen with Piétrain pigs but genetically it was not understood how to most profitably use these traits in breeding programmes.

[0051] Furthermore, the invention provides a transgenic animal, sperm and an embryo derived thereof, comprising a synthetic parentally imprinted QTL or functional fragment thereof as provided by the invention, i.e., it is provided by the invention to introduce a favourable recombinant allele; for example, to introduce the oestrogen receptor locus related to increased litter size of an animal homozygously in a parentally imprinted region of a grandparent animal (for example, the father of a hybrid sow if the region was paternally imprinted and the grandparent was a boar); to introduce a favourable fat-related allele or muscle mass-related recombinant allele in a paternally imprinted region, and so on. Recombinant alleles that are interesting or favourable from the maternal side are often the ones that have opposite effects to alleles from the paternal side. For example, in meat animals such as pigs, recombinant alleles linked with meat quality traits such as intramuscular fat or muscle mass could be fixed in the dam lines while recombinant alleles linked with reduced backfat

could be fixed in the sire lines. Other desirable combinations are, for example, fertility and/or milk yield in the female line with growth rates and/or muscle mass in the male lines.

[0052] The invention is further explained in the detailed description without limiting the invention.

#### Example 1: Wild Boar x Large White intercrosses

#### Methods

[0053] Isolation of an IGF2 BAC clone and fluorescent *in situ* hybridisation (FISH). IGF2 primers (F:5'- GGCAAGTTCTTCCGCTAATGA-3' (SEQ. ID. NO: \_\_\_\_ ) and R:5' - GCACCGCAGAATTACGACAA-3' (SEQ. ID. NO: \_\_\_\_)) for PCR amplification of a part of the last exon and 3'UTR were designed on the basis of a porcine IGF2 cDNA sequence (GenBank X56094). The primers were used to screen a porcine BAC library and the clone 253G10 was isolated. Crude BAC DNA was prepared as described<sup>24</sup>. The BAC DNA was linearized with *EcoRV* and purified with QIAEXII (QIAGEN GmbH, Germany). The clone was labeled with biotin-14-dATP using the GIBCO-BRL Bionick labeling system (BRL18246-015). Porcine metaphase chromosomes were obtained from pokeweed (Seromed) stimulated lymphocytes using standard techniques. The slides were aged for two days at room temperature and then kept at -20°C until use. FISH analysis was carried out as previously described<sup>25</sup>. The final concentration of the probe in the hybridisation mix was 10 ng/μl. Repetitive sequences were suppressed with standard concentrations of porcine genomic DNA. After post-hybridisation washing, the biotinylated probe was detected with two layers of avidin-FITC (Vector A-2011). The chromosomes were counterstained with 0.3 mg/ml DAPI (4, 6-Diamino-2-phenylindole; Sigma D9542), which produced a G-banding like pattern. No posthybridisation banding was needed, since chromosome 2 is easily recognized without banding. A total of 20 metaphase spreads were examined under an Olympus BX-60 fluorescence microscope connected to an IMAC-CCD S30 video camera and equipped with an ISIS 1.65 (Metasystems) software.

Sequence, microsatellite, and linkage analysis.

[0054] About two  $\mu\text{g}$  of linearized and purified BAC DNA was used for direct sequencing with 20 pmoles of primers and BigDye Terminator chemistry (Perkin Elmer, USA). DNA sequencing was done from the 3' end of the last exon towards the 3' end of the UTR until a microsatellite was detected. A primer set (F: 5'-GTTTCTCCTGTACCCACACGCATCCC-3' (SEQ. ID. NO. \_\_\_\_)) and R: 5'-Fluorescein-CTACAAGCTGGGCTCAGGG-3' (SEQ. ID. NO. \_\_\_\_)) was designed for the amplification of the IGF2 microsatellite which is about 250 bp long and located approximately 800 bp downstream from the stop codon. The microsatellite was PCR amplified using fluorescently labeled primers and the genotyping was carried out using an ABI377 sequencer and the GeneScan/Genotyper softwares (Perkin Elmer, USA). Two-point and multipoint linkage analyses were done with the Cri-Map software<sup>26</sup>.

Animals and phenotypic data.

[0055] The intercross pedigree comprised two European Wild Boar males and eight Large White females, 4 F<sub>1</sub> males and 22 F<sub>1</sub> females, and 200 F<sub>2</sub> progeny<sup>1</sup>. The F<sub>2</sub> animals were sacrificed at a live weight of at least 80 kg or at a maximum age of 190 days. Phenotypic data on birth weight, growth, fat deposition, body composition, weight of internal organs, and meat quality were collected; a detailed description of the phenotypic traits is provided by Andersson *et al.*<sup>1</sup> and Andersson-Eklund *et al.*<sup>4</sup>

Statistical analysis.

[0056] Interval mapping for the presence of QTL was carried out using a least squares method developed for the analysis of crosses between outbred lines<sup>27</sup>. The method is based on the assumption that the two divergent lines are fixed for alternative QTL alleles. There are four possible genotypes in the F<sub>2</sub> generation with regards to the grandparental origin of the alleles at each locus. This makes it possible to fit three effects: additive, dominance, and imprinting<sup>2</sup>. The latter is

estimated as the difference between the two types of heterozygotes, the one receiving the Wild Boar allele through an F<sub>1</sub> sire and the one receiving it from an F<sub>1</sub> dam. An F-ratio was calculated using this model (with 3 d.f.) versus a reduced model without a QTL effect for each cM of chromosome 2. The most likely position of a QTL was obtained as the location giving the highest F-ratio. Genome-wise significant thresholds were obtained empirically by a permutation test<sup>28</sup> as described<sup>2</sup>. The QTL model including an imprinting effect was compared with a model without imprinting (with 1 d.f.) to test whether the imprinting effect was significant.

[0057] The statistical models also included the fixed effects and covariants that were relevant for the respective traits; see Andersson-Eklund *et al.*<sup>4</sup> for a more detailed description of the statistical models used. Family was included to account for background genetic effects and maternal effects. Carcass weight was included as a covariant to discern QTL effects on correlated traits, which means that all results concerning body composition were compared at equal weights. Least-squares means for each genotype class at the IGF2 locus were estimated with a single point analysis using Procedure GLM of SAS<sup>29</sup>; the model included the same fixed effects and covariants as used in the interval mapping analyses. The QTL shows a clear parent of origin-specific expression and the map position coincides with that of the insulin-like growth factor II gene (IGF2), indicating IGF2 as the causative gene. A highly significant segregation distortion (excess of Wild Boar-derived alleles) was also observed at this locus. The results demonstrate an important effect of the IGF2 region on postnatal development and it is possible that the presence of a paternally expressed IGF2-linked QTL in humans and in rodent model organisms has so far been overlooked due to experimental design or statistical treatment of data. The study has also important implications for quantitative genetics theory and practical pig breeding.

[0058] IGF2 was identified as a positional candidate gene for this QTL due to the observed similarity between pig chromosome 2p and human chromosome 11p. A genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong, consistent signal on the terminal part of chromosome 2p (Fig. 1). A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IGF2 3'UTR using the BAC clone. A complex microsatellite



was identified about 800 bp downstream of the stop codon; a sequence comparison revealed that this microsatellite is identical to a previously described anonymous microsatellite, *Swc9*<sup>6</sup>. PCR primers were designed and the microsatellite (IGF2ms) was found to be highly polymorphic with three different alleles among the two Wild Boar founders and another two among the eight Large White founders. IGF2ms was fully informative in the intercross as the breed of origin as well as the parent of origin could be determined with confidence for each allele in each F<sub>2</sub> animal.

[0059] A linkage analysis using the intercross pedigree was carried out with IGF2ms and the microsatellites *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. IGF2 was firmly assigned to 2p by highly significant lodscores (e.g.,  $Z=89.0$ ,  $\theta=0.003$  against *Swr2516*). Multipoint analyses, including previously typed chromosome 2 markers<sup>8</sup>, revealed the following order of loci (sex-average map distances in Kosambi cM): *Sw2443*/*Swr2516*-0.3-IGF2-14.9-*Sw2623*-10.3-*Sw256*. No recombinant was observed between *Sw2443* and *Swr2516*, and the suggested proximal location of IGF2 in relation to these loci is based on a single recombinant giving a lodscore support of 0.8 for the reported order. The most distal marker in our previous QTL study, *Sw256*, is located about 25 cM from the distal end of the linkage group.

[0060] QTL analyses of body composition, fatness, meat quality, and growth traits were carried out with the new chromosome 2 map using a statistical model testing for the possible presence of an imprinting effect as expected for IGF2. Clear evidence for a paternally expressed QTL located at the very distal tip of 2p was obtained (Fig. 2; Table 1). The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F<sub>2</sub> population. Large effects on the area of the longissimus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population. The strong imprinting effect observed for all affected traits strongly suggests a single causative locus. The pleiotropic effects on skeletal muscle mass and the size of the heart appear adaptive from a physiological point of view as a larger muscle mass requires a larger cardiac output. The clear paternal expression of this QTL is illustrated

by the least squares means which fall into two classes following the population origin of the paternally inherited allele (Table 1). It is worth noticing though that there was a non-significant trend towards less extreme values for the two heterozygous classes, in particular for the estimated effect on the area of longissimus dorsi. This may be due to chance, but could have a biological explanation, e.g., that there is some expression of the maternally inherited allele or that there is a linked, non-imprinted QTL with minor effects on the traits in question.

[0061] The IGF2-linked QTL and the *FAT1* QTL on chromosome 4 1, 9 are by far the two loci with the largest effect on body composition and fatness segregating in this Wild Boar intercross. The IGF2 QTL controls primarily muscle mass whereas *FAT1* has major effects on fat deposition including abdominal fat, a trait that was not affected by the IGF2 QTL (Fig. 2). No significant interaction between the two loci was indicated and they control a very large proportion of the residual phenotypic variance in the F<sub>2</sub> generation. A model including both QTLs explains 33.1% of the variance for percentage of lean meat in ham, 31.3% for the percentage of lean meat plus bone in back, and 26.2% for average backfat depth (compare with a model including only chromosome 2 effects, Table 1). The two QTLs must have played a major role in the response during selection for lean growth and muscle mass in the Large White domestic pig.

[0062] A highly significant segregation distortion was observed in the IGF2 region (excess of Wild Boar-derived alleles) as shown in Table 1 ( $\chi^2=11.7$ , d.f.=2;  $P=0.003$ ). The frequency of Wild Boar-derived IGF2 alleles was 59% in contrast to the expected 50% and there was twice as many “Wild Boar” as “Large White” homozygotes. This deviation was observed with all three loci at the distal tip and is thus not due to typing errors. The effect was also observed with other loci but the degree of distortion decreased as a function of the distance to the distal tip of the chromosome. Blood samples for DNA preparation were collected at 12 weeks of age and we are convinced that the deviation from expected Mendelian ratios was present at birth as the number of animals lost prior to blood sampling was not sufficient enough to cause a deviation of this magnitude. No other of the more than 250 loci analysed in this pedigree show such a marked segregation distortion (L. Andersson, unpublished). The segregation distortion did not show an imprinting effect, as the frequencies of the two reciprocal types of heterozygotes were identical (Table 1). This does not exclude the possibility that the QTL effects and the segregation distortion are controlled by the same

locus. The segregation distortion may be due to meiotic drive favouring the paternally expressed allele during gametogenesis, as the  $F_1$  parents were all sired by Wild Boar males. Another possibility is that the segregation distortion may be due to codominant expression of the maternal and paternal allele in some tissues and/or during a critical period of embryo development. Biallelic IGF2 expression has been reported to occur to some extent during human development<sup>10, 11</sup> and, interestingly, a strong influence of the parental species background on IGF2 expression was recently found in a cross between *Mus musculus* and *Mus spretus*<sup>12</sup>. It is also interesting that a VNTR polymorphism at the insulin gene, which is very closely linked to IGF2, is associated with size at birth in humans<sup>13</sup>. It is possible that the IGF2-linked QTL in pigs has a minor effect on birth weight, but in our data it was far from significant (Fig. 2) and there was no indication of an imprinting effect.

**[0063]** This study is an advance in the general knowledge concerning the biological importance of the IGF2 locus. The important role of IGF2 for prenatal development is well-documented from knock-out mice<sup>14</sup> as well as from its causative role in the human Beckwith-Wiedemann syndrome<sup>15</sup>. This study demonstrates an important role for the IGF2-region also for postnatal development. It should be stressed that our intercross between outbred populations is particularly powerful to detect QTL with a parent of origin-specific effect on a multifactorial trait. This is because multiple alleles (or haplotypes) are segregating and we could deduce whether a heterozygous  $F_2$  animal received the Wild Boar allele from the  $F_1$  male or female. It is quite possible that the segregation of a paternally expressed IGF2-linked QTL affecting a trait like obesity has been overlooked in human studies or in intercrosses between inbred rodent populations because of experimental design or statistical treatment of data. An imprinting effect cannot be detected in an intercross between two inbred lines as only two alleles are segregating at each locus. Our result has, therefore, significant bearings on the future analysis of the association between genetic polymorphism in the Insulin-IGF2 region and Type I diabetes<sup>16</sup>, obesity<sup>17</sup>, and variation in birth weight<sup>13</sup> in humans, as well as for the genetic dissection of complex traits using inbred rodent models. A major impetus for generating an intercross between the domestic pig and its wild ancestor was to explore the possibilities to map and identify major loci that have responded to selection. We have now showed that two single QTLs on chromosome 2 (this study) and 4<sup>1, 2</sup> explain as much as one third of the phenotypic variance for lean meat content in the  $F_2$  generation. This is a gross deviation from the

underlying assumption in the classical infinitesimal model in quantitative genetics theory, namely, that quantitative traits are controlled by an infinite number of loci, each with an infinitesimal effect. If a large proportion of the genetic difference between two divergent populations (e.g., Wild Boar and Large White) is controlled by a few loci, one would assume that selection would quickly fix QTL alleles with large effects leading to a selection plateau. However, this is not the experience in animal breeding programmes or selection experiments where good, persistent, long-term selection responses are generally obtained, provided that the effective population size is reasonably large<sup>18</sup>. A possible explanation for this paradox is that QTL alleles controlling a large proportion of genetic differences between two populations may be due to several consecutive mutations; this may be mutations in the same gene or at several closely linked genes affecting the same trait. It has been argued that new mutations contribute substantially to long-term selection responses<sup>19</sup>, but the genomic distribution of such mutations is unknown.

**[0064]** The search for a single causative mutation is the paradigm regarding the analysis of genetic defects in mice and monogenic disorders in humans. We propose that this may not be the case for loci that have been under selection for a large number of generations in domestic animals, crops, or natural populations. This hypothesis predicts the presence of multiple alleles at major QTL. It gains some support from our recent characterisation of porcine coat color variation. We have found that both the alleles for dominant white color and for black-spotting differ from the corresponding wild-type alleles by at least two consecutive mutations with phenotypic effects at the *KIT* and *MC1R* loci, respectively<sup>20, 21</sup>. In this context, it is highly interesting that in the accompanying example we have identified a third allele at the IGF2-linked QTL. The effects on muscle mass of the three alleles rank in the same order as the breeds in which they are found, i.e., Piétrain pigs are more muscular than Large White pigs, which in turn have a higher lean meat content than Wild Boars.

**[0065]** There are good reasons to decide that IGF2 is the causative gene for the now reported QTL. Firstly, there is a perfect agreement in map localization (Fig. 2). Secondly, it has been shown that IGF2 is paternally expressed in mice, humans, and now in pigs, like the QTL. There are several other imprinted genes in the near vicinity of IGF2 in mice and humans (*Mash2*, *INS2*, *H19*, *KVLQT1*, *TAPAI/CD81*, and *CDKN1C/p57<sup>KIP2</sup>*) but only IGF2 is paternally expressed in adult tissues<sup>22</sup>. We believe that this locus provides a unique opportunity for molecular characterisation of a QTL. The

clear paternal expression can be used to exclude genes that do not show this mode of inheritance. Moreover, the presence of an allelic series should facilitate the difficult distinction between causative mutations and linked neutral polymorphism. We have already shown that there is no difference in coding sequence between IGF2 alleles from Piétrain and Large White pigs suggesting that the causative mutations occur in regulatory sequences. An obvious step is to sequence the entire IGF2 gene and its multiple promoters from the three populations. The recent report that a VNTR polymorphism in the promoter region of the insulin (*INS*) gene affects IGF2 expression<sup>23</sup> suggests that the causative mutations may be at a considerable distance from the IGF2 coding sequence.

[0066] The results have several important implications for the pig breeding industry. They show that genetic imprinting is not an esoteric academic question but needs to be considered in practical breeding programmes. The detection of three different alleles in Wild Boar, Large White, and Piétrain populations indicates that further alleles at the IGF2-linked QTL segregate within commercial populations. The paternal expression of the QTL facilitates its detection using large paternal half-sib families as the female contribution can be ignored. The QTL is exploited to improve lean meat content by marker-assisted selection within populations or by marker-assisted introgression of favourable alleles from one population to another.

## Example 2: Piétrain x Large White intercrosses

### Methods

[0067] *Pedigree material:* The pedigree material utilised to map QTL was selected from a previously described Piétrain x Large White F2 pedigree comprising > 1,800 individuals<sup>6,7</sup>. To assemble this F2 material, 27 Piétrain boars were mated to 20 Large White sows to generate an F1 generation comprising 456 individuals. 31 F1 boars were mated to unrelated 82 F1 sows from 1984 to 1989, yielding a total of 1862 F2 offspring. F1 boars were mated on average to 7 females, and F1 sows to an average of 2, 7 males. Average offspring per boar was 60 and per sow was 23.

[0068] *Phenotypic information:* (i) *Data collection:* A total of 21 distinct phenotypes were recorded in the F2 generation<sup>6,7</sup>. These included:

- five growth traits: birth weight (g), weaning weight (Kg), grower weight (Kg), finisher weight (Kg) and average daily gain (ADG; Kg/day; grower to finisher period);
- two body proportion measurements: carcass length (cm); and a conformation score (0 to 10 scale; ref.6);
- ten measurements of carcass composition obtained by dissection of the chilled carcasses 24 hours after slaughter. These include measurements of muscularity: % ham (weight hams/carcass weight), % loin (weight loin/carcass weight), % shoulder (weight shoulder/carcass weight), % lean cuts (% ham + % loin + % shoulder); and measurements of fatness: average back-fat thickness (BFT; cm), % backfat (weight backfat/carcass weight), % belly (weight belly/carcass weight), % leaf fat (weight leaf fat/carcass weight), % jowl (weight jowl/carcass weight), and “% fat cuts” (% backfat + % belly + % leaf fat + % jowl).
- four meat quality measurements: pH<sub>LD1</sub> (*Longissimus dorsi* 1 hour after slaughter), pH<sub>LD24</sub> (*Longissimus dorsi* 24 hours after slaughter), pH<sub>GL</sub> (*Gracilis* 1 hour after slaughter) and pH<sub>G24</sub> (*Gracilis* 24 hours after slaughter). (ii) *Data processing*: Individual phenotypes were preadjusted for fixed effects (sire, dam, CRC genotype, sex, year-season, parity) and covariants (litter size, birth weight, weaning weight, grower weight, finisher weight) that proved to significantly affect the corresponding trait. Variables included in the model were selected by stepwise regression.

**[0069] Marker genotyping:** Primer pairs utilised for PCR amplification of microsatellite markers are as described<sup>19</sup>. Marker genotyping was performed as previously described<sup>20</sup>. Genotypes at the *CRC* and *MyoD* loci were determined using conventional methods as described<sup>1,12</sup>. The LAR test for the IGF2 SNP was developed according to Baron et al.<sup>21</sup> using a primer pair for PCR amplification (5'-CCCCTGAACTTGAGGACGAGCAGCC-3' (SEQ. ID. NO. \_\_\_\_); 5'-ATCGCTGTGGGCTGGGTGGGCTGCC-3') (SEQ. ID. NO. \_\_\_\_)) and a set of three primers for the LAR step (5'-FAM- CGCCCCAGCTGCCCCCAG-3' (SEQ. ID. NO. \_\_\_\_); 5' -HEX- CGCCCCAGCTGCCCCCAA-3' (SEQ. ID. NO. \_\_\_\_); 5' -CCTGAGCTGCAGCAGGCCAG-3') (SEQ. ID. NO. \_\_\_\_)).

**[0070] Map construction:** Marker maps were constructed using the TWOPOINT, BUILD and CHROMPIC options of the CRIMAP package<sup>22</sup>. To allow utilisation of this package, full-sib families related via the boar or sow were disconnected and treated independently. By doing so, some

potentially usable information was neglected, yielding, however, unbiased estimates of recombination rates.

[0071] *QTL mapping: (i) Mapping Mendelian QTL:* Conventional QTL mapping was performed using a multipoint maximum likelihood method. The applied model assumed one segregating QTL per chromosome and fixation of alternate QTL alleles in the respective parental lines, Piétrain (P) and Large White (LW). A specific analysis programme had to be developed to account for the missing genotypes of the parental generation, resulting in the fact that the parental origin of the F1 chromosomes could not be determined. Using a typical “interval mapping” strategy, a hypothetical QTL was moved along the marker map using user-defined steps. At each position, the likelihood ( $L$ ) of the pedigree data was computed as:

$$L = \sum_{\varphi=1}^{2^r} \prod_{i=1}^n \sum_{G=1}^4 (P(G|M_i, \theta, \varphi) P(y_i|G))$$

$P$  or right chromosome  $P$ ), there is a total of  $2^r$  combinations for  $r$  F1 parents.

$$\prod_{i=1}^n n \text{ F2}$$

$$\sum_{G=1}^4 \text{ } i\text{th F2 offspring, over the four possible QTL genotypes:}$$

$P/P$ ,  $P/LW$ ,  $LW/P$  and  $LW/LW$

$P(G|M_i, \theta, \varphi)$ : the marker genotype of the  $i$ th F2 offspring and its F1 parents, (ii) the vector of recombination rates between adjacent markers and between the hypothetical QTL and its flanking markers, and (iii)  $\theta$  the considered marker-QTL phase combination of the F1 parents.

Recombination rates and marker linkage phase of F1 parents are assumed to be known when computing this probability. Both were determined using CRIMAP in the map construction phase (see above).

$P(y_i|G)y_i)$  of offspring  $i$ , given the QTL genotype under consideration. This probability is computed from the normal density function:

$$P(y_i|G) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(y_i - \mu_G)^2}{2\sigma^2}}$$

$\mu_G$  is the phenotypic mean of the considered QTL genotype (PP, PL, LP or LL) and  $\sigma^2$  the residual variance.  $\sigma^2$  was considered to be the same for the four QTL genotypic classes. The values of  $\mu_{PP}$ ,  $\mu_{PL} = \mu_{LP}$ ,  $\mu_{LL}$  and  $\sigma^2$  maximizing  $L$  were determined using the GEMINI optimisation routine<sup>23</sup>. The likelihood obtained under this alternative  $H_1$  hypothesis was compared with the likelihood obtained under the null hypothesis  $H_0$  of no QTL, in which the phenotypic means of the four QTL genotypic classes were forced to be identical. The difference between the logarithms of the corresponding likelihoods yields a lodscore measuring the evidence in favour of a QTL at the corresponding map position.

(ii) *Significance thresholds:* Following Lander & Botstein<sup>24</sup>, lodscore thresholds ( $T$ ) associated with a chosen genome-wise significance level were computed such that:

$$\alpha = (C + 9.21GT)\chi^2_2(4.6T)$$

$C$  corresponds to the number of chromosomes (= 19),  $G$  corresponds to the length of the genome in Morgans (= 29), and  $\chi^2_2(4.6T)$  denotes one minus the cumulative distribution function of the chi-squared distribution with 2 d.f. Single point 21n(LR) were assumed to be distributed as a chi-squared distribution with two degrees of freedom, as we were fitting both an additive and dominance component. To account for the fact that we were analysing multiple traits, significance levels were adjusted by applying a Bonferoni correction corresponding to the effective number of independent traits that were analysed. This effective number was estimated at 16 following the approach described



by Spelman et al.<sup>25</sup>. Altogether, this allowed us to set the lodscore threshold associated with an experiment—wise significance level of 5% at 5.8. When attempting to confirm the identified QTL in an independent sample, the same approach was used, however, setting C at 1, G at 25cM and correcting for the analysis of 4.5 independent traits (as only six traits were analysed in this sample). This yielded a lodscore threshold associated with a Type I error of 5% of 2.

(iii) *Testing for an imprinted QTL*: To test for an imprinted QTL, we assumed that only the QTL alleles transmitted by the parent of a given sex would have an effect on phenotype, the QTL alleles transmitted by the other parent being “neutral”. The likelihood of the pedigree data under this hypothesis was computed using equation 1. To compute  $P(y_i | G)$ , however, the phenotypic means of the four QTL genotypes were set at  $\mu_{PP} = \mu_{PL} = \mu_P$  and  $\mu_{LP} = \mu_{LL} = \mu_L$  to test for a QTL for which the paternal allele only is expressed, and  $\mu_{PP} = \mu_{LP} = \mu_P$  and  $\mu_{PL} = \mu_{LL} = \mu_L$  to test for a QTL for which the maternal allele only is expressed. It is assumed in this notation that the first subscript refers to the paternal allele, the second subscript to the maternal allele.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  testing the presence of a Mendelian QTL,  $H_2$  testing the presence of a paternally expressed QTL, and  $H_3$  testing the presence of a maternally expressed QTL.

**[0072]** *RT-PCR*: Total RNA was extracted from skeletal muscle according to Chirgwin et al.<sup>26</sup> RT-PCR was performed using the Gene-Amp RNA PCR Kit (Perkin-Elmer) The PCR products were purified using QiaQuick PCR Purification kit (Qiagen) and sequenced using Dye terminator Cycle Sequencing Ready Reaction (Perkin Elmer) and an ABI373 automatic sequencer. In example 2 we report the identification of a QTL with major effect on muscle mass and fat deposition mapping to porcine 2p1.7. The QTL shows clear evidence for parental imprinting strongly suggesting the involvement of the IGF2 locus.

**[0073]** A Piétrain X Large White intercross comprising 1125  $F_2$  offspring was generated as described<sup>6, 7</sup>. The Large White and Piétrain parental breeds differ for a number of economically important phenotypes. Piétrain are famed for their exceptional muscularity and leanness<sup>8</sup> (Figure 2), while Large Whites show superior growth performance. Twenty-one distinct phenotypes measuring (i) growth performance (5), (ii) muscularity (6), (iii) fat deposition (6), and (iv) meat quality (4) were recorded on all  $F_2$  offspring.

[0074] In order to map QTL underlying the genetic differences between these breeds, we undertook a whole genome scan using microsatellite markers on an initial sample of 677 F<sub>2</sub> individuals. Analysis of pig chromosome 2 using a ML multipoint algorithm revealed highly significant lodscores (up to 20) for six of the 12 phenotypes measuring muscularity and fat deposition at the distal end of the short arm of chromosome 2 (Figure 3a). Positive lodscores were obtained for the remaining six phenotypes, however, not reaching the genome-wide significance threshold (= 5%). To confirm this finding, the remaining sample of 355 F<sub>2</sub> offspring was genotyped for the five most distal 2p markers and QTL analysis performed for the traits yielding the highest lodscores in the first analysis. Lodscores ranged from 2.1 to 7.7, clearly confirming the presence of a major QTL in this region. Table 2 reports the corresponding ML estimates for the three genotypic means as well as the corresponding residual variance.

[0075] Bidirectional chromosome painting establishes a correspondence between SSC2p and HSA1pter-q13<sup>9, 10</sup>. At least two serious candidate genes map to this region in man: the myogenic basic helix-loop-helix factor, *MyoD*, maps to HSA11p15.4, while IGF2 maps to HSA11p15.5. *MyoD* is a well-known key regulator of myogenesis and is one of the first myogenic markers to be switched on during development<sup>11</sup>. A previously described amplified sequence polymorphism in the porcine *MyoD* gene<sup>12</sup> proved to segregate in our F<sub>2</sub> material, which was entirely genotyped for this marker. Linkage analysis positioned the *MyoD* gene in the SW240-SW776 (odds > 1000) interval, therefore, well outside the lod-2 drop off support interval for the QTL (figure 1). IGF2 is known to enhance both proliferation and differentiation of myoblasts *in vitro*<sup>13</sup> and to cause a muscular hypertrophy when overexpressed *in vivo*. Based on a published porcine adult liver cDNA sequence<sup>14</sup>, we designed primer pairs allowing us to amplify the entire IGF2 coding sequence with 222 bp of leader and 280 bp of trailer sequence from adult skeletal muscle cDNA. Piétrain and Large White RT-PCR products were sequenced, indicating that the coding sequences were identical in both breeds and with the published sequence. However, a G A transition was found in the leader sequence corresponding to exon 2 in man (Figure 4). We developed a screening test for this single nucleotide polymorphism (SNP) based on the ligation amplification reaction (LAR), allowing us to genotype our pedigree material. Based on these data, IGF2 was shown to colocalize with the SWC9 microsatellite marker (= 0%), therefore, located at approximately 2 centimorgan from the most likely position of the QTL

and well within the 95% support interval for the QTL (figure 1). Subsequent sequence analysis demonstrated that the microsatellite marker SWC9 is actually located within the 3' UTR of the IGF2 gene. Combined with available comparative mapping data for the PGA and FSH loci, these results suggest the occurrence of an interstitial inversion of a chromosome segment containing *MyoD*, but not IGF2 which has remained telomeric in both species.

[0076] IGF2, therefore, appeared as a strong positional allele having the observed QTL effect. In man and mouse, IGF2 is known to be imprinted and to be expressed exclusively from the paternal allele in several tissues<sup>15</sup>. Analysis of skeletal muscle cDNA from pigs heterozygous for the SNP and/or SWC9 shows that the same imprinting holds in this tissue in the pig as well (Figure 4). Therefore, if IGF2 were responsible for the observed effect, and knowing that only the paternal IGF2 allele is expressed, one can predict that (i) the paternal allele transmitted by F1 boars (P or LW) would have an effect on the phenotype of F2 offspring, (ii) the maternal allele transmitted by F1 sows (P or LW) would have no effect on phenotype of F2 offspring, and (iii) the likelihood of the data would be superior under a model of a bimodal (1:1) F2 population sorted by inherited paternal allele when compared to a conventional "Mendelian" model of a trimodal (1:2:1) F2 population. The QTL mapping programmes were adapted in order to allow testing of the corresponding hypotheses.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  as testing for the presence of a Mendelian QTL,  $H_2$  as testing for the presence of a paternally expressed QTL, and  $H_3$  as testing for the presence of a maternally expressed QTL.

[0077] Figure 3 summarizes the obtained results. Figures 3a, 3b and 3c respectively show the lodscore curves corresponding to  $\log_{10} (H_2/H_0)$ ,  $\log_{10} (H_3/H_0)$  and  $\log_{10} (H_2/H_1)$ . It can be seen that very significant lodscores are obtained when testing for the presence of a paternally expressed QTL, while there is no evidence at all for the segregation of a QTL when studying the chromosomes transmitted by the sows. Also, the hypothesis of a paternally expressed QTL is significantly more likely ( $\log_{10} (H_2/H_1) > 3$ ) than the hypothesis of a "Mendelian" QTL for all examined traits. The fact that the same tendency is observed for all traits indicates that it is likely the same imprinted gene that is responsible for the effects observed on the different traits. Table 2 reports the ML phenotypic means for the F2 offspring sorted by inherited paternal QTL allele. Note that when performing the analysis under a model of a mendelian QTL, the Piétrain and Large White QTL alleles appeared to

behave in an additive fashion, the heterozygous genotype exhibiting a phenotypic mean corresponding exactly to the midpoint between the two homozygous genotypes. This is exactly what one would predict when dealing with an imprinted QTL as half of the heterozygous offspring are expected to have inherited the P allele from their sire, the other half the LW allele.

[0078] These data, therefore, confirmed our hypothesis of the involvement of an imprinted gene expressed exclusively from the paternal allele. The fact that the identified chromosomal segment coincides precisely with an imprinted domain documented in man and mice strongly implicates the orthologous region in pigs. At least seven imprinted genes mapping to this domain have been documented (IGF2, Ins2, H19, Mash2, P57<sup>KIP2</sup>, K<sub>v</sub>LQTL1 and TDAG51) (ref. 15 and Andrew Feinberg, personal communication). Amongst these, only IGF2 and Ins2 are paternally expressed. While we cannot exclude that the observed QTL effect is due to an as of yet unidentified imprinted gene in this region, its reported effects on myogenesis *in vitro* and *in vivo*<sup>13</sup> strongly implicate IGF2. Particularly the muscular hypertrophy observed in transgenic mice overexpressing IGF2 from a muscle-specific promotor are in support of this hypothesis (Nadia Rosenthal, personal communication). Note that allelic variants of the *INS* VNTR have recently been shown to be associated with size at birth in man<sup>16</sup>, and that the same VNTR has been shown to affect the level of IGF2 expression<sup>17</sup>.

[0079] The observation of the same QTL effect in a Large White x Wild Boar intercross indicates the existence of a series of at least three distinct functional alleles. Moreover, preliminary evidence based on marker-assisted segregation analysis points towards residual segregation at this locus within the Piétrain population (data not shown). The occurrence of an allelic series might be invaluable in identifying the causal polymorphisms which - based on the quantitative nature of the observed effect - are unlikely to be gross gene alterations but rather subtle regulatory mutations.

[0080] The effects of the identified QTL on muscle mass and fat deposition are truly major, being of the same magnitude of those reported for the *CRC* locus<sup>6,7</sup> though apparently without the associated deleterious effects on meat quality. We estimate that both loci jointly explain close to 50% of the Piétrain versus Large White breed difference for muscularity and leanness. Understanding the parent-of-origin effect characterising this locus will allow for its optimal use in breeding programmes.

Indeed, today, half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit the unfavourable Large White allele causing considerable loss.

**[0081]** The QTL described in this work is the second example of a gene affecting muscle development in livestock species that exhibits a non-mendelian inheritance pattern. Indeed, we have previously shown that the callipyge locus (related to the qualitative trait wherein muscles are doubled) is characterised by polar overdominance in which only the heterozygous individuals that inherit the CLPG mutation from their sire express the double-muscling phenotype<sup>5</sup>. This demonstrates that parent-of-origin effects affecting genes underlying production traits in livestock might be relatively common.

### Example 3:

Generating a reference sequence of IGF2 and flanking loci in the pig.

**[0082]** The invention provides an imprinted QTL with a major effect on muscle mass mapping to the IGF2 locus in the pig, and use of the QTL as a tool in marker-assisted selection. To fine tune this tool for marker-assisted selection, as well as to further identify a causal mutation, we have further generated a reference sequence encompassing the entire porcine IGF2 sequence as well as that from flanking genes.

**[0083]** To achieve this, we screened a porcine BAC library with IGF2 probes and identified two BACs. BAC-PIGF2-1 proved to contain the INS and IGF2 genes, while BAC-PIGF2-2 proved to contain the IGF2 and H19 genes. The NotI map as well as the relative position of the two BACs is shown in Figure 5. BAC-PIGF2-1 was shotgun sequenced using standard procedures and automatic sequencers. The resulting sequences were assembled using standard software yielding a total of 115 contigs. The corresponding sequences are reported in figure 6. Similarity searches were performed between the porcine contigs and the orthologous sequences in humans. Significant homologies were detected for 18 contigs and are reported in Figure 7.

**[0084]** For BAC-PIGF2-2, the 24 Kb NotI fragment not present in BACPIGF2-1 was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers.

Resulting sequences were assembled using the Phred-Phrap-Consed programme suit, yielding seven distinct contigs (figure 8). The contig sequences were aligned with the corresponding orthologous human sequences using the compare and dotplot programmes of the GCG suite. Figure 9 summarizes the corresponding results.

Example 4: Identification of DNA sequence polymorphisms in the IGF2 and flanking loci.

**[0085]** Based on the reference sequence obtained as described in Example 1, we resequenced part of the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals, allowing identification of DNA sequence polymorphisms such as reported in figure 10.

## REFERENCES

Literature cited with example 1

1. Andersson, L. *et al.* Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science* 263, 1771-1774 (1994).
2. Knott, S.A. *et al.* Multiple marker mapping of quantitative trait loci in a cross between outbred wild boar and Large White pigs. *Genetics* 149, 1069-1080 (1998).
3. Edfors-Lilja, I. *et al.* Mapping quantitative trait loci for immune capacity in the pig. *Journal of Immunology* 161, 829-835 (1998).
4. Andersson-Eklund, L. *et al.* Mapping quantitative trait loci for carcass and meat quality traits in a wild boar x Large White intercross. *Journal of Animal Science* 76, 694-700 (1998).
5. Fronicke, L., Chowdhary, B.P., Scherthan, H. & Gustavsson, I. A comparative map of the porcine and human genomes demonstrates ZOO-FISH and gene mapping-based chromosomal homologies. *Mamm Genome* 7, 285-90 (1996).
6. Alexander, L.J. *et al.* Physical assignments of 68 porcine cosmids and lambda clones containing microsatellites. *Mammalian Genome* 7, 368-372 (1996).

7. Rohrer, G.A. et al. A comprehensive map of the porcine genome. *Genome Research* 6, 371-391 (1996).
8. Marklund, L. et al. A comprehensive linkage map of the pig based on a wild pig-Large White intercross. *Anim Genet* 27, 255-69 (1996).
9. Marklund, L., Nyström, P.E., Stern, S., Andersson-Eklund, L. & Andersson, L. Quantitative trait loci for fatness and growth on pig chromosome 4. *Heredity* In press (1998).
10. Ohlsson, R., Hedborg, F., Holmgren, L., Walsh, C. & Ekström, T.J. Overlapping patterns of IGF2 and H19 expression during human development: biallelic IGF2 expression correlates with a lack of H19 expression. *Development* 120, 361-368 (1994).
11. Ekström, T.J., Cui, H., Li, X. & Ohlsson, R. Promoter-specific IGF2 imprinting status and its plasticity during human liver development. *Development* 121, 309-316 (1995).
12. Hemberger, M. et al. H19 and IGF2 are expressed and differentially imprinted in neuroectoderm-derived cells in the mouse brain. *Dev. Genes Evol.* 208, 393-402 (1998).
13. Dunger, D.B. et al. Association of the INS VNTR with size at birth. *Nature Genetics* 19, 98-100 (1998).



14. DeChiara, T.M., Robertson, E.J. & Efstratiadis, A. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* 64, 849-859 (1991).
15. Sun, F.L., Dean, W.L., Kelsey, G., Allen, N.D. & Reik, W. Transactivation of IGF2 in a mouse model of Beckwith-Wiedemann syndrome. *Nature* 389, 809-815 (1997).
16. Davies, J.L. *et al.* A genome-wide search for human type 1 diabetes susceptibility genes. *Nature* 371, 130-136 (1994).
17. O'Dell, S.D. *et al.* ApaI polymorphism in insulin-like growth factor II (IGF2) gene and weight in middle-aged males. *International Journal of Obesity* 21, 822-825 (1997).
18. Falconer, D.S. & Mackay, T.F.C. *Introduction to Quantitative Genetics*, (Longman, England, 1996).
19. Hill, W.G. Rates of change in quantitative traits from fixation of new mutations. *Proc Natl Acad Sci U S A* 79, 142-145 (1982).
20. Marklund, S. *et al.* Molecular basis for the dominant white phenotype in the domestic pig. *Genome Research* 8, 826-833 (1998).
21. Kijas, J.M.H. *et al.* Melanocortin receptor 1 (*MC1R*) mutations and coat color in the pig. *Genetics* In press(1998).

22. Beechey, C.V. personal communication (1998).
23. Paquette, J., Giannoukakis, N., Polychronakos, C., Vafiadis, P. & Deal, C. The *INS* 5' variable number of tandem repeats is associated with IGF2 expression in humans. *Journal of Biological Chemistry* 273, 14158-14164 (1998).
24. Sambrook, J., Fritsch, E.F. & Maniatis, T. *Molecular cloning: A laboratory manual.*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989).
25. Chowdhary, B.P., de la Sena, C., Harbitz, I., Eriksson, L. & Gustavsson, I. FISH on metaphase and interphase chromosomes demonstrates the physical order of the genes for GPI, CRC, and LIPE in pigs. *Cytogenetics Cell Genetics* 71, 175-178 (1995).
26. Green, P., Falls, K. & Crook, S. *Documentation for CRI-MAP, version 2.4.*, (Washington University School of Medicine, St. Louis, MO, 1990).
27. Haley, C.S., Knott, S.A. & Elsen, J.M. Mapping quantitative trait loci in crosses between outbred lines using least squares. *Genetics* 136, 1195-1207 (1994).
28. Churchill, G.A. & Doerge, R.W. Empirical threshold values for quantitative trait mapping. *Genetics* 138, 963-971 (1994).
29. Anonymous. *SAS version 6.10*, (SAS Institute Inc., Cary, NC., 1990).

References used with example 2:

1. Fuji, J.; Otsu, K.; Zorzato, F.; Deleon, S.; Khanna, V.K.; Weiler, J.E. O'Brien, P.J.; MacLennan, D.H. (1991). Identification of a mutation in the porcine ryanodine receptor associated with malignant hyperthermia. *Science* 253: 448-451.
2. MacLennan, D.H. & Phillips, M.S. (1993). Malignant hyperthermia. *Science* 256:789-794.
3. Grobet, L.; Royo Martin, L.J.; Poncelet, D.; Pirottin, D.; Brouwers, B.; Riquet, J.; Schoeberlein, A.; Dunner, S.; Ménéssier, F.; Massabanda, J.; Fries, R.; Hanset, R.; Georges, M. (1997) A deletion in the myostatin gene causes double muscling in cattle. *Nature Genetics* 17:71-74.
4. Andersson, L.; Haley, C.S.; Ellegren, H.; Knott, S.A.; Johansson, M.; Andersson, K.; Andersson-Eklund, L.; Edfors-Lilja, I.; Fredholm, M.; Hansson, I.; Hakansson, J.; Lundström, K. (1994). Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science* 263:1771-1774.
5. Cockett, N.; Jackson, S.; Shaw, T.; Farnir, F.; Berghmans, S.; Snowden, G.; Nielsen, D.; Georges, M. (1996). Polar overdominance at the ovine callipyge locus. *Science* 273:236-238.
6. Hanset, R.; Dasnois, C.; Scalais, S.; Michaux, C.; Grobet, L. (1995). Genotypes at the locus for halothane sensitivity and performance in a Piétrain x Large White F2. *Genet. Sel. Evol.* 27: 63-76.
7. Hanset, R.; Dasnois, C.; Scalais, S.; Michaux, C.; Grobet, L. (1995). Introgression into the Piétrain genome of the normal allele at the locus for halothane sensitivity. *Genet. Sel. Evol.* 27: 77-88.

8. Olivier, L.; Lauvergne, J.J. (1967). A study of the inheritance of the muscular hypertrophy of the Piétrain pig: preliminary results. *Annales de Médecine Vétérinaire* 111: 104-109.
9. Rettenberger, G.; Klett, C.; Zechner, U.; Kunz, J.; Vogel, W.; Hameister, H. (1995). Visualisation of the conservation of synteny between humans and pigs by heterologous chromosome painting. *Genomics* 26: 372-378.
10. Goureau, A.; Yerle, M.; Schmitz, A.; Riquet, J.; Milan, D.; Pinton, P.; Frelat, G.; Gellin, J. (1996). Human and porcine correspondence of chromosome segments using bidirectional chromosome painting. *Genomics* 36:252-262.
11. Yun, K.; Wold, B. (1996). Skeletal muscle determination and differentiation: story of a core regulatory network and its context. *Current Opinion in Cell Biology* 8:877-889.
12. Knoll, A.; Nebola, M.; Dvorak, J.; Cepica, S. (1997). Detection of a DdeI PCR RFLP within intron 1 of the porcine MYOD1(MYF3) locus. *Animal Genetics* 28, 308-322.
13. Florini, J.R.; Ewton, D.Z.; McWade, F.J. (1995). IGFs, muscle growth, and myogenesis. *Diabetes Review* 3:73-92.
14. Catchpole, I.R.; Engström, W. (1990). Nucleotide sequence of a porcine insulin-like growth factor II cDNA. *Nucleic Acids Research* 18(21):6430.

15. Feil, R.; Moore, T.F.; Oswald, J.; Walter, J.; Sun, F.; Reik, W. (1997). The imprinted insulin like growth factor 2 gene. Pp70 In Genomic Imprinting. Eds. Reik & Surani. IRL Press at Oxford University Press..
16. Dunger, D.B.; Ong, K.K.L.; Huxtable, S.J.; Sherriff, A.; Woods, K.A.; Ahmed, M.L.; Golding, J.; Pembrey, M.E.; Ring, S.; the AISPAC study team, Bennett, S.T.; Todd, J.A. (1998). Association of the INS VNTR with size at birth. *Nature Genetics* 19: 98-100.
17. Paquette, J.; Giannoukakis, N.; Polychronakos, C.; Vafiadis, P.; Deal, C. (1998). The INS 5' variable number of tandem repeats is associated with IGF2 expression in humans. *J. Biol Chem* 273 (23):14158-14164.
18. Andersson-Eklund, L.; Marklund, L.; Lundström, K.; Haley, C.S.; Andersson, K.; Hansson, I.; Moller, M.; Andersson, L. (1998). Mapping Quantitative Trait Loci for carcass and meat quality traits in a Wild Boar x Large White intercross. *J. Anim. Sci.* 76:694-700.
19. Rohrer, G.A.; Alexander, L.J.; Hu, Z.; Keele, J.W.; Smith, T.P.; Beattie, C.W. (1996). A comprehensive map of the porcine genome. *Genome Research*, in the press.
20. Georges, M.; Nielsen, D.; Mackinnon, M.; Mishra, A.; Okimoto, R.; Pasquino, A.T.; Sargeant, L.S.; Sorensen, A.; Steele, M.R.; Zhao, X.; Womack, J.E.; Hoeschele, I. (1995). Mapping quantitative trait loci controlling milk production by exploiting progeny testing. *Genetics* 139: 907-920.

21. Baron, H.; Fung, S.; Aydin, A.; Bahring, S.; Luft, F.C.; Schuster, H. (1996). Oligonucleotide ligation assay (OLA) for the diagnosis of familial hypercholesterolemia. *Nat. Biotechnol.* 14(10):1279-1282.
22. Lander, E.; Green, P. (1987) Construction of multilocus genetic linkage maps in humans. *Proceedings of National Academy of Science (USA)* 84: 2363-2367.
23. Lalouel, J.M. (1983). Optimization of functions. *Contrib. Epidemiol. Biostat.* 4:235—259.
24. Lander, E.S. & Botstein, D. (1989). Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185-199.
25. Spelman, R.L.; Coppieters, W.; Karim, L.; van Arendonk, J.A.M.; Bovenhuis, H. (1996). Quantitative trait loci analysis for five milk production traits on chromosome six in the dutch Holstein-Friesian population. *Genetics* 144:1799-1808.
26. Chirgwin, J.M.; Przybyla, A.E.; MacDonald, R.J.; Rutter, W.J. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18:5294-5299.

Table 1 Summary of QTL analysis for pig chromosome 2 in a Wild Boar/Large White intercross<sup>1</sup>

Trait	F ratio <sup>2</sup>	Map position <sup>3</sup>	Percent of variance <sup>4</sup>	Least squares means <sup>5</sup>	$L^P/W^M$
$L^P/L^M$	QTL	Imprintin g		$W^P/W^M$	$W^P/L^M$
<u>Body composition traits</u>					
Lean meat in ham, %	24.4***	0	30.6	63.6 <sup>a</sup>	66.4 <sup>b</sup>
Lean meat mass in ham, kg	18.1***	1	24.3	4.69 <sup>a</sup>	4.94 <sup>b</sup>
Lean meat + bone in back, %	12.2**	0	17.4	66.3 <sup>a</sup>	69.3 <sup>b</sup>
Longissimus muscle area, cm <sup>2</sup>	10.3**	1	15.4	31.9 <sup>a</sup>	34.5 <sup>b</sup>
<u>Fatness traits</u>					
Average backfat depth, mm	7.1*	0	10.4	27.2 <sup>a</sup>	25.5 <sup>b</sup>
<u>Weight of internal organs</u>					
Heart, gram	9.7**	0	14.4	226 <sup>a</sup>	238 <sup>b</sup>
<u>Meat quality traits</u>					
Reflectance value, EEL	5.7	1	8.1	18.6 <sup>a</sup>	21.8 <sup>b</sup>

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001

**Table 1, continued**

<sup>1</sup>Only the traits for which the QTL peak was in the IGF2 region (0-10 cM) and the test statistic reaching the nominal significance threshold of  $F=3.9$  are included.

<sup>2</sup>"*QTL*" is the test statistic for the presence of a QTL under a genetic model with additive, dominance, and imprinting effects (3 d.f.) while "Imprinting" is the test statistic for the presence of an imprinting effect (1 d.f.), both obtained at the position of the QTL peak. Genome-wise significance thresholds, estimated by permutation, were used for the QTL test while nominal significance thresholds were used for the Imprinting test.

<sup>3</sup>1n cM from the distal end of 2p; IGF2 is located at 0.3 cM.

<sup>4</sup>The reduction in the residual variance of the  $F_2$  population effected by inclusion of an imprinted QTL at the given position.

<sup>5</sup>Means and standard errors estimated at the IGF2 locus by classifying the genotypes according to the population and parent of origin of each allele. *W* and *L* represent alleles derived from the Wild Boar and Large White founders, respectively; superscripts *P* and *M* represent a paternal and maternal origin, respectively. Figures with different letters (superscript a or b) are significantly different at least at the 5% level, and most of them are different at the 1% or 0.1% level.



Table 2 Maximum likelihood phenotypic means for the different F2 genotypes estimated under (i) a model of a mendelian QTL, and (ii) a model assuming an imprinted QTL.

Traits	Mendelian QTL				Imprinted QTL		
	$\mu_{LW/LW}$	$\mu_{LW/P}$	$\mu_{P/P}$	R	$\mu_{PAT/LW}$	$\mu_{PAT/P}$	R
BFT (cm)	2.98	2.84	2.64	0.27	2.94	2.70	0.27
% ham	21.10	21.56	22.15	0.83	21.23	21.95	0.83
% loin	24.96	25.53	26.46	0.91	25.12	26.14	0.93
% lean cuts	65.02	65.96	67.60	1.65	65.23	67.05	1.67
% backfat	6.56	6.02	5.33	0.85	6.43	5.56	0.85
% fat cuts	28.92	27.68	26.66	1.46	28.54	26.99	1.49



## **APPENDIX B**

**(VERSION OF SUBSTITUTE SPECIFICATION EXCLUDING CLAIMS  
WITH MARKINGS TO SHOW CHANGES MADE)**

**(Serial No. 09/868,732)**



PATENT  
Attorney Docket 4951US

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number: \_\_\_\_\_

Date of Deposit with USPS: \_\_\_\_\_

Person making Deposit: \_\_\_\_\_

APPLICATION FOR LETTERS PATENT

for

**SELECTING ANIMALS FOR PARENTALLY IMPRINTED TRAITS**

Inventor:

Andersson, Leif  
Georges, Michel  
Spincemaille, Geert

Attorney:  
Allen C. Turner  
Registration No. 33,041  
TRASKBRITT  
P.O. Box 2550  
Salt Lake City, Utah 84110  
(801) 532-1922

## SELECTING ANIMALS FOR PARENTALLY IMPRINTED TRAITS

### TECHNICAL FIELD

[0001] The invention relates to methods to select breeding animals or animals destined for slaughter for having desired genotypic or potential phenotypic properties, in particular related to muscle mass and/or fat deposition.

### BACKGROUND

[0002] Breeding schemes for domestic animals have so far focused on farm performance traits and carcass quality. This has resulted in substantial improvements in traits like reproductive success, milk production, lean/fat ratio, prolificacy, growth rate and feed efficiency. Relatively simple performance test data have been the basis for these improvements, and selected traits were assumed to be influenced by a large number of genes, each of small effect (the infinitesimal gene model). There are now some important changes occurring in this area. First, the breeding goal of some breeding organisations has begun to include meat quality attributes in addition to the “traditional” production traits. [Secondly]Second, evidence is accumulating that current and new breeding goal traits may involve relatively large effects (known as major genes), as opposed to the infinitesimal model that has been relied on so far.

[0003] Modern DNA-technologies provide the opportunity to exploit these major genes, and this approach is a very promising route for the improvement of meat quality, especially since direct meat quality assessment is not viable for potential breeding animals. Also for other traits such as lean/fat ratio, growth rate and feed efficiency, modern DNA technology can be very effective. Also these traits are not always easy to measure in the living animal.

[0004] The evidence for several of the major genes was originally obtained using segregation analysis, i.e., without any DNA marker information. Afterwards, molecular studies were performed to detect the location of these genes on the genetic map. In practice, and except for alleles of very large effect, DNA studies are required to dissect the genetic nature of most traits of economic importance. DNA markers can be used to localise genes or alleles responsible for qualitative traits like coat color, and they can also be used to detect genes or alleles with substantial effects on

quantitative traits like growth rate, IMF<sub>2</sub> etc. In this case, the approach is referred to as QTL (quantitative trait locus) mapping, wherein a QTL comprises at least a part of the nucleic acid genome of an animal where genetic information capable of influencing said quantitative trait (in said animal or in its offspring) is located. Information at the DNA level can not only help to fix a specific major gene in a population, but also assist in the selection of a quantitative trait which is already selected for. Molecular information in addition to phenotypic data can increase the accuracy of selection and therefore the selection response.

[0005] Improving meat quality or carcass quality is not just about changing levels of traits like tenderness or marbling, but it is also about increasing uniformity. The existence of major genes provides excellent opportunities for improving meat quality because it allows large steps to be made in the desired direction. [Secondly, it]It will help to reduce variation, since we can fix relevant genes in our products. Another aspect is that selecting for major genes allows differentiation for specific markets. Studies are underway in several species, particularly, pigs, sheep, deer and beef cattle.

[0006] In particular, intense selection for meat production has resulted in animals with extreme muscularity and leanness in several livestock species. In recent years it has become feasible to map and clone several of the genes causing these phenotypes, paving the way towards more efficient [marker assisted]marker-assisted selection, targeted drug development ([performance enhancing]performance-enhancing products) and transgenesis. Mutations in the ryanodine receptor (Fuji et al, 1991; MacLennan and Phillips, 1993) and myostatin (Grobet et al, 1997; Kambadur et al, 1997; McPherron and Lee, 1997) have been shown to cause muscular hypertrophies in pigs and cattle respectively, while genes with major effects on muscularity and/or fat deposition have, for instance, been mapped to pig chromosome 4 (Andersson et al, 1994) and sheep chromosome 18 (Cocket et al, 1996).

#### DISCLOSURE OF THE INVENTION

[0007] However, although there have been successes in identifying QTLs, the information is currently of limited use within commercial breeding programmes. Many workers in this field conclude that it is necessary to identify the particular genes underlying the QTL. This is a substantial task, as the QTL region is usually relatively large and may contain many genes. Identification of the

relevant genes from the many that may be involved thus remains a significant hurdle in farm animals.

**[0008]** The invention provides a method for selecting a domestic animal for having desired genotypic or potential phenotypic properties comprising testing [said]the animal for the presence of a parentally imprinted qualitative or quantitative trait locus (QTL). Herein, a domestic animal is defined as an animal being selected or having been derived from an animal having been selected for having desired genotypic or potential phenotypic properties.

**[0009]** Domestic animals provide a rich resource of genetic and phenotypic variation[,]; traditionally, domestication involves selecting an animal or its offspring for having desired genotypic or potential phenotypic properties. This selection process has in the past century been facilitated by growing understanding and utilisation of the laws of Mendelian inheritance. One of the major problems in breeding programmes of domestic animals is the negative genetic correlation between reproductive capacity and production traits. This is, for example, the case in cattle (a high milk production generally results in slim cows and bulls), poultry[,] (broiler lines have a low level of egg production and layers have generally very low muscle growth), pigs (very prolific sows are, in general, fat and have comparatively less meat) or sheep (high prolific breeds have low carcass quality and vice versa). The invention now provides that knowledge of the parental imprinting character of various traits allows to select, for example, sire lines homozygous for a paternally imprinted QTL, for example, linked with muscle production or growth; the selection for such traits can thus be less stringent in dam lines in favour of the reproductive quality. The phenomenon of genetic or parental imprinting has never been utilised in selecting domestic animals[,]; it was never considered feasible to employ this elusive genetic characteristic in practical breeding programmes. The invention provides a breeding programme, wherein knowledge of the parental imprinting character of a desired trait, as demonstrated herein, results in a breeding programme, for example[ in], a BLUP programme, with a modified animal model. This increases the accuracy of the breeding value estimation and speeds up selection compared to conventional breeding programmes. Until now, the effect of a parentally imprinted trait in the estimation of a conventional BLUP programme was neglected; using and understanding the parental character of the desired trait, as provided by the invention, allows selecting on parental imprinting, even without DNA testing. For example, selecting genes characterised by paternal imprinting is provided to help increase uniformity; a (terminal) parent homozygous for the

“good or wanted” alleles will pass them to all offspring, regardless of the other parent’s alleles, and the offspring will all express the desired parent’s alleles. This results in more uniform offspring. Alleles that are interesting or favourable from the maternal side [or]are often the ones that have opposite effects to alleles from the paternal side. For example, in meat animals such as pigs, alleles linked with meat quality traits such as [inta-muscular]intramuscular fat or muscle mass could be fixed in the dam lines while alleles linked with reduced [back fat]backfat could be fixed in the sire lines. Other desirable combinations are, for example, fertility and/or milk yield in the female line with growth rates and/or muscle mass in the male lines.

[0010] In a preferred embodiment, the invention provides a method for selecting a domestic animal for having desired genotypic or potential phenotypic properties comprising testing a nucleic acid sample from [said]the animal for the presence of a parentally imprinted quantitative trait locus (QTL). A nucleic acid sample can, in general, be obtained from various parts of the animal’s body by methods known in the art. Traditional samples for the purpose of nucleic acid testing are blood samples or skin or mucosal surface samples, but samples from other tissues can be used as well[,]; in particular, sperm samples, oocyte or embryo samples can be used. In such a sample, the presence and/or sequence of a specific nucleic acid, be it DNA or RNA, can be determined with methods known in the art, such as hybridisation or nucleic acid amplification or sequencing techniques known in the art. The invention provides testing such a sample for the presence of nucleic acid wherein a QTL or allele associated therewith is associated with the phenomenon of parental imprinting, for example, where it is determined whether a paternal or maternal allele of said QTL is capable of being predominantly expressed in [said]the animal.

[0011] The purpose of breeding programmes in livestock is to enhance the performances of animals by improving their genetic composition. In essence, this improvement accrues by increasing the frequency of the most favourable alleles for the genes influencing the performance characteristics of interest. These genes are referred to as QTL. Until the beginning of the nineties, genetic improvement was achieved via the use of biometrical methods, but without molecular knowledge of the underlying QTL.

[0012] Since the beginning of the nineties and due to recent developments in genomics, it is conceivable to identify the QTL underlying a trait of interest. The invention now provides identifying

and using parentally imprinted QTLs which are useful for selecting animals by mapping quantitative trait loci. Again, the phenomenon of genetic or paternal imprinting has never been utilised in selecting domestic animals[,]; it was never considered feasible to employ this elusive genetic characteristic in practical breeding programmes. For example, Kovacs and Kloting (Biochem. Mol. Biol. Int. 44:399-405, 1998), where parental imprinting is not mentioned, and not suggested, found linkage of a trait in female rats, but not in males, suggesting a possible sex specificity associated with a chromosomal region, which, of course, excludes parental imprinting, a phenomenon wherein the imprinted trait of one parent is preferably but genderaspecifically expressed in his or her offspring.

[0013] The invention provides the initial localisation of a parentally imprinted QTL on the genome by linkage analysis with genetic markers, and the actual identification of the parentally imprinted gene(s) and causal mutations therein. Molecular knowledge of such a parentally imprinted QTL allows for more efficient breeding designs herewith provided. Applications of molecular knowledge of parentally imprinted QTLs in breeding programmes include: [marker assisted]marker-assisted segregation analysis to identify the segregation of functionally distinct, parentally imprinted QTL alleles in the populations of interest, [marker assisted]marker-assisted selection (MAS) performed within lines to enhance genetic response by increasing selection accuracy[, ] or selection intensity or by reducing the generation interval using the understanding of the [phenomonon]phenomenon of parental imprinting, [marker assisted]marker-assisted introgression (MAI) to efficiently transfer favourable parentally imprinted QTL alleles from a donor to a recipient population, genetic engineering of the identified parentally imprinted QTL and genetic modification of the breeding stock using transgenic technology, and development of performance enhancing products using targeted drug development exploiting molecular knowledge of said QTL.

## BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

--Legends to the Figures

[0014] Fig. 1: Test statistic curves obtained in QTL analyses of chromosome 2 in a Wild Boar/Large White intercross. The graph plots the F ratio testing the hypothesis of a single QTL at



a given position along the chromosome for the traits indicated. The marker map with the distances between markers in Kosambi centiMorgan is given on the X-axis. The horizontal lines represent genome-wise significant ( $P < 0.05$ ) and suggestive levels for the trait lean meat in ham; similar significance thresholds were obtained for the other traits.

[0015] Figure 2: Piétrain pig with characteristic muscular hypertrophy.

[0016] [Figure 3]Figures 3A-3C: Lodscore curves obtained in a Piétrain x Large White intercross for six phenotypes measuring muscle mass and fat deposition on pig chromosome 2. The most likely positions of the *[Igf2]*IGF2 and *MyoD* genes determined by linkage analysis with respect to the microsatellite marker map are shown.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  as testing for the presence of a Mendelian QTL,  $H_2$  as testing for the presence of a paternally expressed QTL, and  $H_3$  as testing for the presence of a maternally expressed QTL. [3a]3A:  $\log_{10}(H_1/H_0)$ , [3b]3B:  $\log_{10}(H_2/H_0)$ , [3c]3C:  $\log_{10}(H_3/H_0)$ .

[0017] Figure 4: A. Structure of the human *[Igf2]*IGF2 gene according to ref. 17, with aligned porcine adult liver cDNA sequence as reported in ref. 16. The position of the *nt241(G-A)* transition and *Swc9* microsatellite are shown. B. The corresponding markers were used to demonstrate the monoallelic (paternal) expression of *[Igf2]*IGF2 in skeletal muscle and liver of 10-week old fetuses. PCR amplification of the *nt421(G-A)* polymorphism and *Swc9* microsatellite from genomic DNA clearly shows the heterozygosity of the fetus, while only the paternal allele is detected in liver cDNA (*nt421(G-A)* and *Swc9*) and muscle cDNA (*Swc9*). The absence of RT-PCR product for *nt421(G-A)* [from ]in fetal muscle points towards the absence of mRNA including exon 2 in this tissue. Parental origin of the [foetal]fetal alleles was determined from the genotypes of the sire and dam (data not shown).

[0018] Figure 5: A NotI restriction map showing the relative position of BAC-PIGF2-1 (comprising INS and IGF2 genes), and BAC-PIGF2-2 (comprising IGF2 and H19 genes).

[0019] Figure 6: Nucleic acid sequences of contig 1 to contig 115 derived from BAC-PIGF2-1 which was shotgun sequenced using standard procedures and automatic sequencers.

[0020] Figure 7: Similarity between porcine contigs of figure 6 and orthologous sequences in human.

[0021] Figure 8: Nucleic acid sequences of contig 1 to contig 7 derived from BAC-PIGF2-2, (the 24 Kb NotI fragment not present in BAC-PIGF2-1) which was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers.

[0022] Figure 9: Similarity between porcine contigs of figure 8 and orthologous sequences in human.

[0023] Figure 10: DNA sequence polymorphisms in the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals.--

### DETAILED DESCRIPTION OF THE INVENTION

[0024] The inventors undertook two independent experiments to determine the practical use of parental imprinting of a QTL.

[0025] In a first experiment, performed in a previously described Piétrain x Large White intercross, the likelihood of the data were computed under a model of paternal (paternal allele only expressed) and maternal imprinting (maternal allele only expressed) and compared with the likelihood of the data under a model of a conventional "Mendelian" QTL. The results strikingly demonstrated that the QTL was indeed paternally expressed, the QTL allele (Piétrain or Large White) inherited from the F<sub>1</sub> sow having no effect whatsoever on the carcass quality and quantity of the F<sub>2</sub> offspring. It was seen that very significant lodscores were obtained when testing for the presence of a paternally expressed QTL, while there was no evidence at all for the segregation of a QTL when studying the chromosomes transmitted by the sows. The same tendency was observed for all traits, showing that the same imprinted gene is responsible for the effects observed on the different traits. Table 1 reports the maximum likelihood (ML) phenotypic means for the F<sub>2</sub> offspring sorted by inherited paternal QTL allele.

[0026] In a second experiment performed in the Wild Boar X Large White intercross, QTL analyses of body composition, fatness, meat quality, and growth traits [was]were carried out with the chromosome 2 map using a statistical model testing for the presence of an imprinting effect. Clear evidence for a paternally expressed QTL located at the very distal tip of 2p was obtained (Fig. 2; [Table]Table 1). The clear paternal expression of a QTL is illustrated by the least squares means which fall into two classes following the population origin of the paternally inherited allele (Table 1).

For a given paternally imprinted QTL, implementation of [marker assisted]marker-assisted segregation analysis, selection (MAS) and introgression (MAI)[,] can be performed using genetic markers that are linked to the QTL, using genetic markers that are in linkage disequilibrium with the QTL, or using the actual causal mutations within the QTL.

[0027] Understanding the parent-of-origin effect characterising a QTL allows for its optimal use in breeding programmes. Indeed, [marker assisted]marker-assisted segregation analysis under a model of parental imprinting will yield better estimates of QTL allele effects. Moreover, it allows for the application of specific breeding schemes to optimally exploit a QTL. In one embodiment of the invention, the most favourable QTL alleles would be fixed in breeding animal lines and, for example, used to generate commercial, crossbred males by [marker assisted]marker-assisted selection (MAS, within lines) and [marker assisted]marker-assisted introgression (MAI, between lines). In another embodiment, the worst QTL alleles would be fixed in the animal lines used to generate commercial crossbred females by MAS (within lines) and MAI (between lines).

[0028] In a preferred embodiment of the invention, [said]the animal is a pig. Note, for example, that the invention provides the insight that today half of the offspring from commercially popular [Piértrain]Piértrain x Large White crossbred boars inherit an unfavourable Large White muscle mass QTL as provided by the invention, causing considerable loss, and the invention now, for example, provides the possibility to select the better half of the population in that respect. However, it is also possible to select commercial sow lines enriched [with the in]within the boars unfavourable alleles, allowing [to equip ]the sows to be equipped with other alleles more desirable for, for example, reproductive purposes.

[0029] In a preferred embodiment of a method provided by the invention, said QTL is located at a position corresponding to a QTL located at chromosome 2 in the pig. For example, it is known to form comparative mapping data between pig and human, including bidirectional chromosome painting[,]and that SSC2p is homologous to HSA1pter-q13<sup>11, 12</sup>. HSA1pter-q13 is known to harbour a cluster of imprinted genes: IGF2, INS2, H19, MAH2, P57<sup>KIP2</sup>, K<sub>v</sub>LQTL1, Tapa1/CD81, Orct12, Impt1 and Ip1. The cluster of imprinted genes located in HSA1pter-q13 is characterised by 8 maternally expressed genes: H19, MASH2, P57<sup>KIP2</sup>, K<sub>v</sub>LQTL1, TAPA1/CD81, ORCTL2, IMPT1 and IPI, and two paternally expressed genes: IGF2 and INS. However, Johanson et al. (Genomics

25:682-690, 1995) and Reik et al. (Trends in Genetics, 13:330-334, 1997) show that the whereabouts of these loci in various animals are not clear. For example, the HSA11 and MMU7 loci do not correspond among each other, the MMU7 and the SSC2 loci do not correspond, whereas the HSA11 and SSC2 loci seem to correspond, and no guidance is given where one or more of, for example, the [above identified]above-identified parentally expressed individual genes are localised on the three species' chromosomes.

**[0030]** Other domestic animals, such as cattle, sheep, poultry and fish, having similar regions in their genome harbouring such a cluster of imprinted genes or QTLs, the invention herewith provides use of these orthologous regions of other domestic animals in applying the phenomenon of parental imprinting in breeding programmes. In pigs, said cluster is mapped at around position 2p1.7 of chromosome 2[,]; however, a method as provided by the invention employing (fragments of) said maternally or paternally expressed orthologous or homologous genes or QTLs [are]is advantageously used in other animals as well as for breeding and selecting purposes. For example, a method is provided wherein said QTL is related to the potential muscle mass and/or fat deposition, preferably with limited effects on other traits such as meat quality and daily gain of [said]the animal or wherein said QTL comprises at least a part of an insulin-like growth factor-2 (IGF2) allele. Reik et al. (Trends in Genetics, 13:330-334, 1997) explain that this gene in humans is related to Beckwith-Wiedemann syndrome, an apparently parentally imprinted disease syndrome most commonly seen with human [foetuses]fetuses, where the gene has an important role in prenatal development. No relationship is shown or suggested with postnatal development relating to muscle development or fatness in (domestic) animals.

**[0031]** In a preferred embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7. In particular, the invention relates to the use of genetic markers for the telomeric end of pig chromosome 2p in marker selection (MAS) of a parentally imprinted Quantitative Trait Locus (QTL) affecting carcass yield and quality in pigs. Furthermore, the invention relates to the use of genetic markers associated with the IGF2 locus in MAS in pigs, such as polymorphisms and microsatellites and other characterising nucleic acid sequences shown herein, such

as shown in figures 4 to 10. In a preferred embodiment, the invention provides a QTL located at the distal tip of *Sus scrofa* chromosomes 2 with effects on [varies]various measurements of carcass quality and quantity, particularly muscle mass and fat deposition.

**[0032]** In a first experiment, a QTL mapping analysis was performed in a Wild Boar X Large White intercross counting 200  $F_2$  individuals. The  $F_2$  animals were sacrificed at a live [eight]weight of at least 80 kg or at a maximum age of 190 days. Phenotypic data on birth weight, growth, fat deposition, body composition, weight of internal organs, and meat quality were collected; a detailed description of the phenotypic traits are provided by Andersson et al.<sup>1</sup> and Andersson-Eklund et al.<sup>4</sup>

**[0033]** A QTL (without any significant effect on back-fat thickness) at an unspecified locus on the proximal end of chromosome 2 with moderate effect on muscle mass, and located about 30cM away from the parentally imprinted QTL reported here, was previously reported by the inventors; whereas the QTL as now provided has a very large effect, explaining at least 20-30% of variance, making the QTL of the present invention commercially very attractive, which is even more so because the present QTL is parentally imprinted. The marker map of chromosome 2p was improved as part of this invention by adding microsatellite markers in order to cover the entire chromosome arm. The following microsatellite markers were used: *Swc9*, *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. QTL analyses of body composition, fatness, meat quality, and growth traits were carried out with the new chromosome 2 map. Clear evidence for a QTL located at the very distal tip of 2p was obtained (Fig. 1; Table 1). The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the  $F_2$  population. Large effects on the area of the longissimus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population.

**[0034]** In a second experiment, QTL mapping was performed in a Piétrain X Large White intercross comprising 1125  $F_2$  offspring. The Large White and Piétrain parental breeds differ for a number of economically important phenotypes. Piétrains are famous for their exceptional muscularity

and leanness<sup>10</sup> (Figure 2), while Large Whites show superior growth performance. Twenty-one distinct phenotypes measuring growth performance (5), muscularity (6), fat deposition (6), and meat quality (4), were recorded on all F<sub>2</sub> offspring. In order to map QTL underlying the genetic differences between these breeds, the inventors undertook a whole genome scan using microsatellite markers on an initial sample of 677 F<sub>2</sub> individuals. The following microsatellite marker map was used to analyse chromosome 2; [.] SW2443, SWC9 and SW2623, SWR2516- (0,20) -SWR783- (0,29) -SW240- (0,20) -SW776- (0,08) -S0010- (0, 04) -SW1695-(0,36) -SWR308. Analysis of pig chromosome 2 using a Maximum Likelihood multipoint algorithm[,] revealed highly significant lodscores (up to 20) for three of the six phenotypes measuring muscularity (% lean cuts, % ham, % loin) and three of the six phenotypes measuring fat deposition (back-fat thickness (BFT), % backfat, % fat cuts) at the distal end of the short arm of chromosome 2 (Figure 1). Positive lodscores were obtained in the corresponding chromosome region for the remaining six muscularity and fatness phenotypes, however, not reaching the experiment-wise significance threshold[)] ( $\alpha=5\%$ ). There was no evidence for an effect of the corresponding QTL on growth performance (including birth weight) or recorded meat quality measurements (data not shown). To confirm this finding, the remaining sample of 355 F<sub>2</sub> offspring was genotyped for the four most distal 2p markers and QTL analysis performed for the traits yielding the highest lodscores in the first analysis. Lodscores ranged from 2.1 to 7.7, clearly confirming the presence of a major QTL in this region. Table 2 reports the corresponding ML estimates for the three genotypic means as well as the residual variance. Evidence based on [marker assisted] marker-assisted segregation analysis points towards residual segregation at this locus within the Piétrain population.

[0035] These experiments, therefore, clearly indicated the existence of a QTL with a major effect on carcass quality and quantity on the telomeric end of pig chromosome arm 2p[;], the likely existence of an allelic series at this QTL with at least three alleles: Wild-Boar < Large White < Piétrain, and possibly more given the observed segregation within the Piétrain breed.

[0036] The effects of the identified QTL on muscle mass and fat deposition are truly major, being of the same magnitude of those reported for the *CRC* locus though apparently without the associated deleterious effects on meat quality. We estimate that both loci jointly explain close to 50% of the Piétrain versus Large White breed difference for muscularity and leanness. The QTL had very

large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F<sub>2</sub> population. Large effects on the area of the longissimus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL, when compared to the Wild Boar allele, was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population. The strong imprinting effect observed for all affected traits shows that a single causative locus is involved. The pleiotropic effects on skeletal muscle mass and the size of the heart appear adaptive from a physiological point of view as a larger muscle mass requires a larger cardiac output.

[0037] In a further embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7., wherein said QTL comprises at least a part of a *Sus scrofa* insulin-like growth factor-2 (IGF2) allele or a [genomic]genomic area closely related thereto, such as polymorphisms and microsatellites and other characterising nucleic acid sequences shown herein, such as shown in figures 4 to 10. The important role of IGF2 for prenatal development is well-documented from knockout mice as well as from its causative role in the human Beckwith-Wiedemann syndrome. This invention demonstrates an important role for the IGF2-region, also for postnatal development.

[0038] To show the role of [Igf2]IGF2, the inventors performed the following three experiments:

[0039] A genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong consistent signal on the terminal part of chromosome 2p.

[0040] A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IGF2 3'UTR using the BAC clone. A complex microsatellite was identified about 800bp downstream of the stop

codon; a sequence comparison revealed that this microsatellite was identical to a previously described anonymous microsatellite, *Swc9*<sup>6</sup>. This marker was used in the initial QTL mapping experiments and its location on the genetic map correspond with the most likely position of the QTL both in the Piétrain X Large White and in the Large White x Wild Boar pedigree.

[0041] Analysis of skeletal muscle and liver cDNA from 10-week old [foetuses]fetuses heterozygous for a nt241 (G-A) transversion in the second exon of the porcine [IGFII]IGF2 gene and SWC9[,] shows that the [IGFII]IGF2 gene is imprinted in these tissues in the pig as well and only expressed from the paternal allele.

[0042] Based on a published porcine adult liver cDNA sequence<sup>16</sup>, the inventors designed primer pairs allowing [to amplify]amplification of the entire [*IgfII*]IGF2 coding sequence with 222 bp of leader and 280 bp of trailer sequence from adult skeletal muscle cDNA. Piétrain and Large White RT-PCR products were sequenced[ indication], indicating that the coding sequences are identical in both breeds and with the published sequence. However, a G↔A transition was found in the leader sequence corresponding to exon 2 in man. Following conventional nomenclature, this polymorphism will be referred to as *nt241* (G-A). We developed a screening test for this single nucleotide polymorphism 9(SNP) based on the ligation amplification reaction (LAR), allowing us to genotype our pedigree material. Based on these data, [*IgfII*]IGF2 was shown to colocalize with the SWC9 microsatellite marker (θ=0%), therefore virtually coinciding with the most likely position of the QTL, and well within the 95% support interval for the QTL. Subsequent sequence analysis demonstrated that the microsatellite marker SWC9 is actually located within the 3'UTR of the [*IgfII*]IGF2 gene.

[0043] As previously mentioned, the knowledge of this QTL provides a method for the selection of animals such as pigs with improved carcass merit. Different embodiments of the invention are envisaged, including: [marker assisted]marker-assisted segregation analysis to identify the segregation of functionally distinct QTL alleles in the populations of interest; [marker assisted]marker-assisted selection (MAS) performed within lines to enhance genetic response by increasing selection accuracy[,]or selection intensity or by reducing the generation interval; [marker assisted]and marker-assisted introgression (MAI) to efficiently transfer favourable QTL alleles from



a donor to a recipient population, thereby enhancing genetic response in the recipient population. Implementation of embodiments [marker assisted] of marker-assisted segregation analysis, selection (MAS) and introgression (MAI)[,] can be performed using genetic markers that are linked to the QTL[;], genetic markers that are in linkage disequilibrium with the QTL, and the actual causal mutations within the QTL.

**[0044]** In a further embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a Sus scrofa chromosome 2 mapping at position 2p1.7., wherein said QTL is paternally expressed, i.e., is expressed from the paternal allele. In man and mouse, *[Igf]IGF2* is known to be imprinted and to be expressed exclusively from the paternal allele in several tissues. Analysis of skeletal muscle cDNA from pigs heterozygous for the SNP and/or SWC9[, ] shows that the same imprinting holds in the pig as well. Understanding the parent-of-origin effect characterising the QTL as provided by the invention now allows for its optimal use in breeding programmes. Indeed, today, half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit the unfavourable Large White allele, causing considerable loss. Using a method as [provide] provided by the invention avoids this problem.

**[0045]** The invention furthermore provides an isolated and/or recombinant nucleic acid or functional fragment derived thereof comprising a parentally imprinted quantitative trait locus (QTL) or fragment thereof capable of being predominantly expressed by one parental allele. Having such a nucleic acid as provided by the invention available allows constructing transgenic animals wherein favourable genes are capable of being exclusively or predominantly expressed by one parental allele, thereby equipping the offspring of [said] the animal homozygous for a desired trait with desired properties related to that parental allele that is expressed.

**[0046]** In a preferred embodiment, the invention provides an isolated and/or recombinant nucleic acid or fragment derived thereof comprising a synthetic parentally imprinted quantitative trait locus (QTL) or functional fragment thereof derived from at least one chromosome. Synthetic herein describes a parentally expressed QTL wherein various elements are combined that originate from distinct locations from the genome of one or more animals. The invention provides recombinant

nucleic acid wherein sequences related to parental imprinting of one QTL are combined with sequences relating to genes or favourable alleles of a second QTL. Such a gene construct is favourably used to obtain transgenic animals wherein the second QTL has been equipped with paternal imprinting, as opposed to the inheritance pattern in the native animal from which the second QTL is derived. Such a second QTL can, for example, be derived from the same chromosome where the parental imprinting region is located, but can also be derived from a different chromosome from the same or even a different species. In the pig, such a second QTL can, for example, be related to an oestrogen receptor (ESR)-gene (Rothschild et al., PNAS, 93, 201-201, 1996) or a FAT-QTL (Andersson, Science, 263, 1771-1774, 1994) for example, derived from [an other]another pig chromosome, such as chromosome 4. A second or further QTL can also be derived from another (domestic) animal or a human.

[0047] The invention furthermore provides an isolated and/or recombinant nucleic acid or functional fragment derived thereof at least partly corresponding to a QTL of a pig located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7 wherein said QTL is related to the potential muscle mass and/or fat deposition of said pig and/or wherein said QTL comprises at least a part of a *Sus scrofa* insulin-like growth factor-2 (IGF2) allele, preferably at least spanning a region between INS and H19, or preferably derived from a domestic pig, such as a [Pietrain]Piétrain, Meishan, Duroc, Landrace or Large White, or from a Wild Boar. For example, a genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAG clone gave a strong, consistent signal on the terminal part of chromosome 2p. A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IGF2 3'UTR using the BAC clone. A complex microsatellite was identified about 800 bp downstream of the stop codon; a sequence comparison revealed that this microsatellite is identical to a previously described anonymous microsatellite, *Swc9*. PGR primers were designed and the microsatellite (IGF2ms) was found to be highly polymorphic with three different alleles among the two Wild Boar founders and another two among the eight Large White founders. IGF2ms was fully informative in the intercross as the breed of origin as well as the parent of origin could be determined with confidence for each allele in each F<sub>2</sub> animal.

[0048] A linkage analysis using the intercross pedigree was carried out with IGF2ms and the microsatellites *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. IGF2 was firmly assigned to 2p by highly significant [lod scores]lodscores (e.g.  $Z=89.0$ ,  $\theta=0.003$  against *Swr2516*). Multipoint analyses, including previously typed chromosome 2 markers, revealed the following order of loci (sex-average map distances in Kosambi cM): *Sw2443*/*Swr2516*-0.3-IGF2-14.9-*Sw2623*-10.3-*Sw256*. No recombinant was observed between *Sw2443* and *Swr2516*, and the suggested proximal location of IGF2 in relation to these loci is based on a single recombinant giving a [lod score]lodscore support of 0.8 for the reported order. The most distal marker in our previous QTL study, *Sw256*, is located about 25 cM from the distal end of the linkage group.

[0049] The invention furthermore provides use of a nucleic acid or functional fragment derived thereof according to [the invention in ]a method according to the invention. In a preferred embodiment, use of a method according to the invention is provided to select a breeding animal or animal destined for slaughter, or embryos or semen derived from these animals, for having desired genotypic or potential phenotypic properties. In particular, the invention provides such use wherein said properties are related to muscle mass and/or fat deposition. The QTL as provided by the invention may be exploited or used to improve, for example, lean meat content or back-fat thickness by [marker assisted]marker-assisted selection within populations or by [marker assisted]marker-assisted introgression of favourable alleles from one population to another. Examples of [marker assisted]marker-assisted selection using the QTL as provided by the invention are use of [marker assisted]marker-assisted segregation analysis with linked markers or with markers in disequilibrium to identify functionally distinct QTL alleles. Furthermore, identification of a causative mutation in the QTL is now possible, again leading to identify functionally distinct QTL alleles. Such functionally distinct QTL alleles located at the distal tip of chromosome 2p with large effects on skeletal muscle mass, the size of the heart, and on back-fat thickness are also provided by the invention. The observation of a similar QTL effect in a Large White x Wild Boar as well as in a Piétrain x Large White intercross provides proof of the existence of a series of at least three distinct functional alleles. Moreover, preliminary evidence based on [marker assisted]marker-assisted segregation analysis points towards residual segregation at this locus within the Piétrain population (data not shown). The occurrence of an allelic series as provided by the invention allows identifying causal polymorphisms

which - based on the quantitative nature of the observed effect - are unlikely to be gross gene alterations but rather subtle regulatory mutations. The effects on muscle mass of the three alleles rank in the same order as the breeds in which they are found, i.e., Piétrain pigs are more muscular than Large White pigs that in turn have higher lean meat content than Wild Boars. The invention furthermore provides use of the alleles as provided by the invention [for ]within line selection or for [marker assisted]marker-assisted introgression using linked markers, markers in disequilibrium or alleles comprising causative mutations.

[0050] The invention furthermore provides an animal selected by using a method according to the invention. For example, a pig characterised in being homozygous for an allele in a QTL located at a Sus scrofa chromosome 2 mapping at position 2p1.7 can now be selected and is thus provided by the invention. Since said QTL is related to the potential muscle mass and/or fat deposition of said pig and/or said QTL comprises at least a part of a Sus scrofa insulin-like growth factor-2 (IGF2) allele, it is possible to select promising pigs to be used for breeding or to be slaughtered. In particular, an animal according to the invention which is a male is provided. Such a male, or its sperm or an embryo derived thereof, can advantageously be used in breeding animals for creating breeding lines or for finally breeding animals destined for slaughter. In a preferred embodiment of such use as provided by the invention, a male, or its sperm, deliberately selected for being homozygous for an allele causing the extreme muscular [hyperthrophy]hypertrophy and leanness, is used to produce offspring heterozygous for such an allele. Due to said allele's paternal expression, said offspring will also show the favourable traits, for example, related to muscle mass, even if the parent female has a different genetic background. Moreover, it is now possible to positively select the female(s) for having different traits, for example, related to fertility, without having a negative effect on the muscle mass trait that is inherited from the allele from the selected male. For example, earlier such males could occasionally be seen with Piétrain pigs but genetically it was not understood how to most profitably use these traits in breeding programmes.

[0051] Furthermore, the invention provides a transgenic animal, sperm and an embryo derived thereof, comprising a synthetic parentally imprinted QTL or functional fragment thereof as provided by the invention, i.e., it is provided by the invention to introduce a favourable recombinant allele; for example, to introduce the oestrogen receptor locus related to increased litter size of an

animal homozygously in a parentally imprinted region of a grandparent animal (for example, the father of a hybrid sow if the region was paternally imprinted and the grandparent was a boar); to introduce a favourable fat-related allele or muscle mass-related recombinant allele in a paternally imprinted region, and so on. Recombinant alleles that are interesting or favourable from the maternal side [or]are often the ones that have opposite effects to alleles from the paternal side. For example, in meat animals such as pigs, recombinant alleles linked with meat quality traits such as [intramuscular]intramuscular fat or muscle mass could be fixed in the dam lines while recombinant alleles linked with reduced [back fat]backfat could be fixed in the sire lines. Other desirable combinations are, for example, fertility and/or milk yield in the female line with growth rates and/or muscle mass in the male lines.

**[0052]** The invention is further explained in the detailed description without limiting the invention.

#### Example 1: Wild Boar x Large White intercrosses

#### Methods

**[0053]** Isolation of an IGF2 BAC clone and fluorescent *in situ* hybridisation (FISH). IGF2 primers (F:5'- GGCAAGTTCTTCCGCTAATGA-3' (SEQ. ID. NO: \_\_\_\_ ) and R:5' - GCACCGCAGAATTACGACAA-3' (SEQ. ID. NO: \_\_\_\_)) for PCR amplification of a part of the last exon and 3'UTR were designed on the basis of a porcine IGF2 cDNA sequence (GenBank X56094). The primers were used to screen a porcine BAC library and the clone 253G10 was isolated. Crude BAC DNA was prepared as described<sup>24</sup>. The BAC DNA was linearized with *EcoRV* and purified with QIAEXII (QIAGEN GmbH, Germany). The clone was labeled with biotin-14-dATP using the GIBCO-BRL Bionick labeling system (BRL18246-015). Porcine metaphase chromosomes were obtained from pokeweed (Seromed) stimulated lymphocytes using standard techniques. The slides were aged for two days at room temperature and then kept at -20°C until use. FISH analysis was carried out as previously described<sup>25</sup>. The final concentration of the probe in the hybridisation mix was 10 ng/μl. Repetitive sequences were suppressed with standard concentrations

of porcine genomic DNA. After post-hybridisation washing, the biotinylated probe was detected with two layers of avidin-FITC (Vector A-2011). The chromosomes were counterstained with 0.3 mg/ml DAPI (4, 6-Diamino-2-phenylindole; Sigma D9542), which produced a G-banding like pattern. No posthybridisation banding was needed, since chromosome 2 is easily recognized without banding. A total of 20 metaphase spreads were examined under an Olympus BX-60 fluorescence microscope connected to an IMAC-CCD S30 video camera and equipped with an ISIS 1.65 (Metasystems) software.

Sequence, microsatellite, and linkage analysis.

**[0054]** About two  $\mu\text{g}$  of linearized and purified BAC DNA was used for direct sequencing with 20 pmoles of primers and BigDye Terminator chemistry (Perkin Elmer, USA). DNA sequencing was done from the 3' end of the last exon towards the 3' end of the UTR until a microsatellite was detected. A primer set (F: 5'-GTTTCTCCTGTACCCACACGCATCCC-3' (SEQ. ID. NO: \_\_\_\_ ) and R: 5' -Fluorescein- CTACAAGCTGGGCTCAGGG-3') (SEQ. ID. NO: \_\_\_\_ ) was designed for the amplification of the IGF2 microsatellite which is about 250 bp long and located approximately 800 bp downstream from the stop codon. The microsatellite was PCR amplified using fluorescently labeled primers and the genotyping was carried out using an ABI377 sequencer and the GeneScan/Genotyper softwares (Perkin Elmer, USA). Two-point and multipoint linkage [analysis]analyses were done with the Cri-Map software<sup>26</sup>.

Animals and phenotypic data.

**[0055]** The intercross pedigree comprised two European Wild Boar males and eight Large White females, 4 F<sub>1</sub> males and 22 F<sub>1</sub> females, and 200 F<sub>2</sub> progeny<sup>1</sup>. The F<sub>2</sub> animals were sacrificed at a live weight of at least 80 kg or at a maximum age of 190 days. Phenotypic data on birth weight, growth, fat deposition, body composition, weight of internal organs, and meat quality were collected; a detailed description of the phenotypic traits [are]is provided by Andersson *et al.*<sup>1</sup> and Andersson-Eklund *et al.*<sup>4</sup>

Statistical analysis.

[0056] Interval mapping for the presence of QTL [were]was carried out [with]using a least squares method developed for the analysis of crosses between outbred lines<sup>27</sup>. The method is based on the assumption that the two divergent lines are fixed for alternative QTL alleles. There are four possible genotypes in the F<sub>2</sub> generation [as]was regards to the grandparental origin of the alleles at each locus. This makes it possible to fit three effects: additive, dominance, and imprinting<sup>2</sup>. The latter is estimated as the difference between the two types of heterozygotes, the one receiving the Wild Boar allele through an F<sub>1</sub> sire and the one receiving it from an F<sub>1</sub> dam. An F-ratio was calculated using this model (with 3 d.f.) versus a reduced model without a QTL effect for each cM of chromosome 2. The most likely position of a QTL was obtained as the location giving the highest F-ratio. Genome-wise [significance]significant thresholds were obtained empirically by a permutation test<sup>28</sup> as described<sup>2</sup>. The QTL model including an imprinting effect was compared with a model without imprinting (with 1 d.f.) to test whether the imprinting effect was significant.

[0057] The statistical models also included the fixed effects and [covariates]covariants that were relevant for the respective traits; see Andersson-Eklund *et al.*<sup>4</sup> for a more detailed description of the statistical models used. Family was included to account for background genetic effects and maternal effects. Carcass weight was included as a [covariate]covariant to discern QTL effects on correlated traits, which means that all results concerning body composition were compared at equal weights. Least-squares means for each genotype class at the IGF2 locus were estimated with a single point analysis using Procedure GLM of SAS<sup>29</sup>; the model included the same fixed effects and [covariates]covariants as used in the interval mapping analyses. The QTL shows a clear parent of origin-specific expression and the map position coincides with that of the insulin-like growth factor II gene (IGF2), indicating IGF2 as the causative gene. A highly significant segregation distortion (excess of Wild Boar-derived alleles) was also observed at this locus. The results demonstrate an important effect of the IGF2 region on postnatal development and it is possible that the presence of a paternally expressed IGF2-linked QTL in humans and in rodent model organisms has so far been

overlooked due to experimental design or statistical treatment of data. The study has also important implications for quantitative genetics theory and practical pig breeding.

**[0058]** IGF2 was identified as a positional candidate gene for this QTL due to the observed similarity between pig chromosome 2p and human chromosome 11p. A genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong, consistent signal on the terminal part of chromosome 2p (Fig. 1). A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IGF2 3'UTR using the BAC clone. A complex microsatellite was identified about 800 bp downstream of the stop codon; a sequence comparison revealed that this microsatellite is identical to a previously described anonymous microsatellite, *Swc9*<sup>6</sup>. PCR primers were designed and the microsatellite (IGF2ms) was found to be highly polymorphic with three different alleles among the two Wild Boar founders and another two among the eight Large White founders. IGF2ms was fully informative in the intercross as the breed of origin as well as the parent of origin could be determined with confidence for each allele in each F<sub>2</sub> animal.

**[0059]** A linkage analysis using the intercross pedigree was carried out with IGF2ms and the microsatellites *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. IGF2 was firmly assigned to 2p by highly significant [lod scores] lodscores (e.g.,  $Z=89.0$ ,  $\theta=0.003$  against *Swr2516*). Multipoint analyses, including previously typed chromosome 2 markers<sup>8</sup>, revealed the following order of loci (sex-average map distances in Kosambi cM): *Sw2443*/*Swr2516*-0.3-IGF2-14.9-*Sw2623*-10.3-*Sw256*. No recombinant was observed between *Sw2443* and *Swr2516*, and the suggested proximal location of IGF2 in relation to these loci is based on a single recombinant giving a [lod score] lodscore support of 0.8 for the reported order. The most distal marker in our previous QTL study, *Sw256*, is located about 25 cM from the distal end of the linkage group.

**[0060]** QTL analyses of body composition, fatness, meat quality, and growth traits were carried out with the new chromosome 2 map using a statistical model testing for the possible presence of an imprinting effect as expected for IGF2. Clear evidence for a paternally expressed QTL located at the very distal tip of 2p was obtained (Fig. 2; Table 1). The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F<sub>2</sub>



population. Large effects on the area of the longissimus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population. The strong imprinting effect observed for all affected traits strongly suggests a single causative locus. The pleiotropic effects on skeletal muscle mass and the size of the heart appear adaptive from a physiological point of view as a larger muscle mass requires a larger cardiac output. The clear paternal expression of this QTL is illustrated by the least squares means which fall into two classes following the population origin of the paternally inherited allele (Table 1). It is worth noticing though that there was a non-significant trend towards less extreme values for the two heterozygous classes, in particular for the estimated effect on the area of longissimus dorsi. This may be due to chance, but could have a biological explanation, e.g., that there is some expression of the maternally inherited allele or that there is a linked, non-imprinted QTL with minor effects on the traits in question.

**[0061]** The IGF2-linked QTL and the *FAT1* QTL on chromosome 4 1, 9 are by far the two loci with the largest effect on body composition and fatness segregating in this Wild Boar intercross. The IGF2 QTL controls primarily muscle mass whereas *FAT1* has major effects on fat deposition including abdominal fat, a trait that was not affected by the IGF2 QTL (Fig. 2). No significant interaction between the two loci was indicated and they control a very large proportion of the residual phenotypic variance in the  $F_2$  generation. A model including both QTLs explains 33.1% of the variance for percentage of lean meat in ham, 31.3% for the percentage of lean meat plus bone in back, and 26.2% for average [back fat]backfat depth (compare with a model including only chromosome 2 effects, Table 1). The two QTLs must have played a major role in the response during selection for lean growth and muscle mass in the Large White domestic pig.

**[0062]** A highly significant segregation distortion was observed in the IGF2 region (excess of Wild Boar-derived alleles) as shown in Table 1 ( $\chi^2=11.7$ , d.f.=2;  $P=0.003$ ). The frequency of Wild Boar-derived IGF2 alleles was 59% in contrast to the expected 50% and there was twice as many “Wild Boar” as “Large White” homozygotes. This deviation was observed with all three loci at the

distal tip and is thus not due to typing errors. The effect was also observed with other loci but the degree of distortion decreased as a function of the distance to the distal tip of the chromosome. Blood samples for DNA preparation were collected at 12 weeks of age and we are convinced that the deviation from expected Mendelian ratios was present at birth as the number of animals lost prior to blood sampling was not sufficient enough to cause a deviation of this magnitude. No other of the more than 250 loci analysed in this pedigree show such a marked segregation distortion (L. Andersson, unpublished). The segregation distortion did not show an imprinting effect, as the frequencies of the two reciprocal types of heterozygotes were identical (Table 1). This does not exclude the possibility that the QTL effects and the segregation distortion are controlled by the same locus. The segregation distortion [maybe] may be due to meiotic drive favouring the paternally expressed allele during gametogenesis, as the F<sub>1</sub> parents were all sired by Wild Boar males. Another possibility is that the segregation distortion may be due to codominant expression of the maternal and paternal allele in some tissues and/or during a critical period of embryo development. Biallelic IGF2 expression has been reported to occur to some extent during human development<sup>10, 11</sup> and, interestingly, a strong influence of the parental species background on IGF2 expression was recently found in a cross between *Mus musculus* and *Mus spretus*<sup>12</sup>. It is also interesting that a VNTR polymorphism at the insulin gene, which is very closely linked to IGF2, is associated with size at birth in humans<sup>13</sup>. It is possible that the IGF2-linked QTL in pigs has a minor effect on birth weight, but in our data it was far from significant (Fig. 2) and there was no indication of an imprinting effect.

[0063] This study is an advance in the general knowledge concerning the biological importance of the IGF2 locus. The important role of IGF2 for prenatal development is well-documented from knock-out mice<sup>14</sup> as well as from its causative role in the human Beckwith-Wiedemann syndrome<sup>15</sup>. This study demonstrates an important role for the IGF2-region also for postnatal development. It should be stressed that our intercross between outbred populations is particularly powerful to detect QTL with a parent of origin-specific effect on a multifactorial trait. This is because multiple alleles (or haplotypes) are segregating and we could deduce whether a heterozygous F<sub>2</sub> animal received the Wild Boar allele from the F<sub>1</sub> male or female. It is quite possible that the segregation of a paternally expressed IGF2-linked QTL affecting a trait like obesity has been overlooked in human studies or in intercrosses between inbred rodent populations because of

experimental design or statistical treatment of data. An imprinting effect cannot be detected in an intercross between two inbred lines as only two alleles are segregating at each locus. Our result has, therefore, significant bearings on the future analysis of the association between genetic polymorphism in the Insulin-IGF2 region and Type I diabetes<sup>16</sup>, obesity<sup>17</sup>, and variation in birth weight<sup>13</sup> in humans, as well as for the genetic dissection of complex traits using inbred rodent models. A major impetus for generating an intercross between the domestic pig and its wild ancestor was to explore the possibilities to map and identify major loci that have responded to selection. We have now showed that two single QTLs on chromosome 2 (this study) and 4<sup>1, 2</sup> explain as much as one third of the phenotypic variance for lean meat content in the F<sub>2</sub> generation. This is a gross deviation from the underlying assumption in the classical infinitesimal model in quantitative genetics theory, namely, that quantitative traits are controlled by an infinite number of loci, each with an infinitesimal effect. If a large proportion of the genetic difference between two divergent populations (e.g., Wild Boar and Large White) is controlled by a few loci, one would assume that selection would quickly fix QTL alleles with large effects leading to a selection plateau. However, this is not the experience in animal breeding programmes or selection experiments where good, persistent, long-term selection responses are generally obtained, provided that the effective population size is reasonably large<sup>18</sup>. A possible explanation for this paradox is that QTL alleles controlling a large proportion of genetic differences between two populations may be due to several consecutive mutations; this may be mutations in the same gene or at several closely linked genes affecting the same trait. It has been argued that new mutations contribute substantially to long-term selection responses<sup>19</sup>, but the genomic distribution of such mutations [are]is unknown.

**[0064]** The search for a single causative mutation is the paradigm [as regards]regarding the analysis of genetic defects in mice and monogenic disorders in humans. We propose that this may not be the case for loci that have been under selection for a large number of generations in domestic animals, crops, or natural populations. This hypothesis predicts the presence of multiple alleles at major QTL. It gains some support from our recent characterisation of porcine coat color variation. We have found that both the alleles for dominant white color and for black-spotting differ from the corresponding wild-type alleles by at least two consecutive mutations with phenotypic effects at the *KIT* and *MC1R* loci, respectively<sup>20, 21</sup>. In this context, it is highly interesting that in the accompanying

example we have identified a third allele at the IGF2-linked QTL. The effects on muscle mass of the three alleles rank in the same order as the breeds in which they are found, i.e., Piétrain pigs are more muscular than Large White pigs [that], which in turn have a higher lean meat content than Wild Boars.

[0065] There are good reasons to decide that IGF2 is the causative gene for the now reported QTL. Firstly, there is a perfect agreement in map localization (Fig. 2). Secondly, it has been shown that IGF2 is paternally expressed in mice, humans, and now in pigs, like the QTL. There are several other imprinted genes in the near vicinity of IGF2 in mice and humans (*Mash2*, *INS2*, *H19*, *KVLQT1*, *TAPAI/CD81*, and *CDKN1C/p57<sup>KIP2</sup>*) but only IGF2 is paternally expressed in adult tissues<sup>22</sup>. We believe that this locus provides a unique opportunity for molecular characterisation of a QTL. The clear paternal expression can be used to exclude genes that do not show this mode of inheritance. Moreover, the presence of an allelic series should facilitate the difficult distinction between causative mutations and linked neutral polymorphism. We have already shown that there is no difference in coding sequence between IGF2 alleles from Piétrain and Large White pigs suggesting that the causative mutations occur in regulatory sequences. An obvious step is to sequence the entire IGF2 gene and its multiple promoters from the three populations. The recent report that a VNTR polymorphism in the promoter region of the insulin (*INS*) gene affects IGF2 expression<sup>23</sup> suggests that the causative mutations may be at a considerable distance from the IGF2 coding sequence.

[0066] The results have several important implications for the pig breeding industry. They show that genetic imprinting is not an esoteric academic question but [need]needs to be considered in practical breeding programmes. The detection of three different alleles in Wild Boar, Large White, and Piétrain populations indicates that further alleles at the IGF2-linked QTL segregate within commercial populations. The paternal expression of the QTL facilitates its detection using large paternal half-sib families as the female contribution can be ignored. The QTL is exploited to improve lean meat content by [marker assisted]marker-assisted selection within populations or by [marker assisted]marker-assisted introgression of favourable alleles from one population to another.

## Example 2: Piétrain x Large White intercrosses

### Methods

[0067] *Pedigree material*: The pedigree material utilised to map QTL was selected from a previously described Piétrain x Large White F2 pedigree comprising > 1,800 individuals<sup>6, 7</sup>. To assemble this F2 material, 27 Piétrain boars were mated to 20 Large White sows to generate an F1 generation comprising 456 individuals. 31 F1 boars were mated to unrelated 82 F1 sows from 1984 to 1989, yielding a total of 1862 F2 offspring. F1 boars were mated on average to 7 females, and F1 sows to an average of 2, 7 males. Average offspring per boar [were]was 60 and per sow was 23.

[0068] *Phenotypic information*: (i) *Data collection*: A total of 21 distinct phenotypes were recorded in the F2 generation<sup>6, 7</sup>. These included:

- five growth traits: birth weight (g), weaning weight (Kg), grower weight (Kg), finisher weight (Kg) and average daily gain (ADG; Kg/day; grower to [finsher]finisher period);
- two body proportion measurements: carcass length (cm); and a conformation score (0 to 10 scale; ref.6);
- ten measurements of carcass composition obtained by dissection of the chilled carcasses 24 hours after slaughter. These include measurements of muscularity: % ham (weight hams/carcass weight), % loin (weight loin/carcass weight), % shoulder (weight shoulder/carcass weight), % lean cuts (% ham + % loin + % shoulder); and measurements of fatness: average back-fat thickness (BFT; cm), % backfat (weight backfat/carcass weight), % belly (weight belly/carcass weight), % leaf fat (weight leaf fat/carcass weight), % jowl (weight jowl/carcass weight), and “% fat cuts” (% backfat + % belly + % [leaf]leaf fat + % jowl).
- four meat quality measurements: pH<sub>LD1</sub> (*Longissimus dorsi* 1 hour after slaughter), pH<sub>LD24</sub> (*Longissimus dorsi* 24 hours after slaughter), pH<sub>G1</sub> (*Gracilis* 1 hour after slaughter) and pH<sub>G24</sub> (*Gracilis* 24 hours after slaughter). (ii) *Data processing*: Individual phenotypes were preadjusted for fixed effects (sire, dam, CRC genotype, sex, year-season, parity) and [covariates]covariants (litter size, birth weight, weaning weight, grower weight, finisher weight) that proved to significantly affect the corresponding trait. Variables included in the model were selected by stepwise regression.

[0069] *Marker genotyping:* Primer pairs utilised for PCR amplification of microsatellite markers are as described<sup>19</sup>. Marker genotyping was performed as previously described<sup>20</sup>. Genotypes at the *CRC* and *MyoD* loci were determined using conventional methods as described<sup>1,12</sup>. The LAR test for the [Igf2]*IGF2* SNP was developed according to Baron et al.<sup>21</sup> using a primer pair for PCR amplification (5'-CCCCTGAACTTGAGGACGAGCAGCC-3' (SEQ. ID. NO: \_\_\_\_); 5'-ATCGCTGTGGGCTGGGTGGGCTGCC-3') (SEQ. ID. NO: \_\_\_\_ and a set of three primers for the LAR step (5'-FAM- CGCCCCAGCTGCCCCCAG-3' (SEQ. ID. NO: \_\_\_\_); 5' -HEX- CGCCCCAGCTGCCCCCAA-3' (SEQ. ID. NO: \_\_\_\_); 5' -CCTGAGCTGCAGCAGGCCAG-3') (SEQ. ID. NO: \_\_\_\_).

[0070] *Map construction:* Marker maps were constructed using the TWOPOINT, BUILD and CHROMPIC options of the CRIMAP package<sup>22</sup>. To allow utilisation of this package, full-sib families related via the boar or sow were disconnected and treated independently. By doing so, some potentially usable information was neglected, yielding, however, unbiased estimates of recombination rates.

[0071] *QTL mapping: (i) Mapping Mendelian QTL:* Conventional QTL mapping was performed using a multipoint maximum likelihood method. The applied model assumed one segregating QTL per chromosome[,] and fixation of alternate QTL alleles in the respective parental lines, Piétrain (P) and Large White (LW). A specific analysis programme had to be developed to account for the missing genotypes of the parental generation, resulting in the fact that the parental origin of the F1 chromosomes could not be determined. Using a typical “interval mapping” strategy, [an]a hypothetical QTL was moved along the marker map using user-defined steps. At each position, the likelihood ( $L$ ) of the pedigree data was computed as:

$$L = \sum_{\varphi=1}^{2^r} \prod_{i=1}^n \sum_{G=1}^4 (P(G|M_p, \theta, \varphi) P(y_i|G))$$

$P$  or right [chromosome]chromosome  $P$ ), there is a total of  $2^r$  combinations for  $r$  F1 parents.

$$\prod_{i=1}^n n \text{ F2}$$

$$\sum_{G=1}^4 \text{ } i\text{th F2 offspring, over the four possible QTL genotypes:}$$

*P/P, P/LW, LW/P and LW/LW*

$P(G|M_i, \theta, \phi)M_i$ : the marker genotype of the  $i$ th F2 offspring and its F1 parents, (ii)  $[r]$  the vector of recombination rates between adjacent markers and between the hypothetical QTL and its flanking markers, and (iii)  $\theta$  the considered marker-QTL phase combination of the F1 parents.

Recombination rates and marker linkage phase of F1 parents are assumed to be known when computing this probability. Both were determined using CRIMAP in the map construction phase (see above).

$P(y_i|G)y_i$  of offspring  $i$ , given the QTL genotype under consideration. This probability is computed from the normal density function:

$$P(y_i|G) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(y_i - \mu_G)^2}{2\sigma^2}}$$

$\mu_G$  is the phenotypic mean of the considered QTL genotype (PP, PL, LP or LL) and  $\sigma^2$  the residual variance.  $\sigma^2$  was considered to be the same for the four QTL genotypic classes. The values of  $\mu_{PP}$ ,  $\mu_{PL} = \mu_{LP}$ ,  $\mu_{LL}$  and  $\sigma^2$  maximizing  $L$  were determined using the GEMINI optimisation routine<sup>23</sup>. The likelihood obtained under this alternative  $H_1$  hypothesis was compared with the likelihood obtained under the null hypothesis  $H_0$  of no QTL, in which the phenotypic means of the four QTL genotypic classes were forced to be identical. The difference between the logarithms of the corresponding

likelihoods yields a lodscore measuring the evidence in favour of a QTL at the corresponding map position.

(ii) *Significance thresholds*: Following Lander & Botstein<sup>24</sup>, lodscore thresholds ( $T$ ) associated with a chosen genome-wide significance level[, ] were computed such that:

$$\alpha = (C+9.21GT)\chi^2_2(4.6T)$$

$C$  corresponds to the number of chromosomes (= 19),  $G$  corresponds to the length of the genome in Morgans (= 29), and  $\chi^2_2(4.6T)$  denotes one minus the cumulative distribution function of the chi-squared distribution with 2 d.f. Single point 21n(LR) were assumed to be distributed as a chi-squared distribution with two degrees of freedom, as we were fitting both an additive and dominance component. To account for the fact that we were analysing multiple traits, significance levels were adjusted by applying a Bonferoni correction corresponding to the effective number of independent traits that were analysed. This effective number was estimated at 16 following the approach described by Spelman et al.<sup>25</sup>. Altogether, this allowed us to set the lodscore threshold associated with an experiment—wise significance level of 5% at 5.8. When attempting to confirm the identified QTL in an independent sample, the same approach was used, however, setting  $C$  at 1,  $G$  at 25cM and correcting for the analysis of 4.5 independent traits (as only six traits were analysed in this sample). This yielded a lodscore threshold associated with a Type I error of 5% of 2.

(iii)[.] *Testing for an imprinted QTL*: To test for an imprinted QTL, we assumed that only the QTL alleles transmitted by the parent of a given sex would have an effect on phenotype, the QTL alleles transmitted by the other parent being “neutral”. The likelihood of the pedigree data under this hypothesis was computed using equation 1. To compute  $P(y_i | G)$ , however, the phenotypic means of the four QTL genotypes were set at  $\mu_{PP} = \mu_{PL} = \mu_P$  and  $\mu_{LP} = \mu_{LL} = \mu_L$  to test for a QTL for which the paternal allele only is expressed, and  $\mu_{PP} = \mu_{LP} = \mu_P$  and  $\mu_{PL} = \mu_{LL} = \mu_L$  to test for a QTL for which the maternal allele only is expressed. It is assumed in this notation that the first subscript refers to the paternal allele, the second subscript to the maternal allele.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  testing the presence of a Mendelian QTL[;],  $H_2$  testing the presence of a paternally expressed QTL, and  $H_3$  testing the presence of a maternally expressed QTL.



[0072] *RT-PCR*: Total RNA was extracted from skeletal muscle according to Chirgwin et al.<sup>26</sup>[.] RT-PCR was performed using the Gene-Amp RNA PCR Kit (Perkin-Elmer) The PCR products were purified using QiaQuick PCR Purification kit (Qiagen) and sequenced using Dye terminator Cycle Sequencing Ready Reaction (Perkin Elmer) and an ABI373 automatic sequencer. In example 2 we report the identification of a QTL with major effect on muscle mass and fat deposition mapping to porcine 2p1.7. The QTL shows clear evidence for parental imprinting strongly suggesting the involvement of the *[Igf]/IGF2* locus.

[0073] A Piétrain X Large White intercross comprising 1125 F<sub>2</sub> offspring was generated as described<sup>6, 7</sup>. The Large White and Piétrain parental breeds differ for a number of economically important phenotypes. Piétrain are famed for their exceptional muscularity and *[leannes<sup>8</sup>]/leanness<sup>8</sup>* (Figure 2), while Large Whites show superior growth performance. Twenty-one distinct phenotypes measuring (i) growth performance (5), (ii) muscularity (6), (iii) fat deposition (6), and (iv) meat quality (4)[.] were recorded on all F<sub>2</sub> offspring.

[0074] In order to map QTL underlying the genetic differences between these breeds, we undertook a whole genome scan using microsatellite markers on an initial sample of 677 F<sub>2</sub> individuals. Analysis of pig chromosome 2 using a ML multipoint algorithm[,] revealed highly significant lodscores (up to 20) for six of the 12 phenotypes measuring muscularity and fat deposition at the distal end of the short arm of chromosome 2 (Figure 3a). Positive lodscores were obtained for the remaining six phenotypes, however, not reaching the genome-wise significance threshold (= 5%). To confirm this finding, the remaining sample of 355 F<sub>2</sub> offspring was genotyped for the five most distal 2p markers and QTL analysis performed for the traits yielding the highest lodscores in the first analysis. Lodscores ranged from 2.1 to 7.7, clearly confirming the presence of a major QTL in this region. Table 2 reports the corresponding ML estimates for the three genotypic means as well as the corresponding residual variance.

[0075] Bidirectional chromosome painting establishes a correspondence between SSC2p and HSA1pter-q13<sup>9, 10</sup>. At least two serious candidate genes map to this region in man: the myogenic basic helix-loop-helix factor, *MyoD*, maps to HSA11p15.4, while *[Igf]/IGF2* maps to HSA11p15.5. *MyoD* is a [well known]well-known key regulator of myogenesis and is one of the first myogenic markers to be switched on during development<sup>11</sup>. A previously described amplified sequence

polymorphism in the porcine *MyoD* gene<sup>12</sup> proved to segregate in our F<sub>2</sub> material, which was entirely genotyped for this marker. Linkage analysis positioned the *MyoD* gene in the SW240-SW776 (odds > 1000) interval, therefore, well outside the lod-2 drop off support interval for the QTL (figure 1). *[Igf]/IGF2* is known to enhance both proliferation and differentiation of myoblasts *in vitro*<sup>13</sup> and to cause a muscular hypertrophy when overexpressed *in vivo*. Based on a published porcine adult liver cDNA sequence<sup>14</sup>, we designed primer pairs allowing us to amplify the entire *[Igf2]/IGF2* coding sequence with 222 bp of leader and 280 bp of trailer sequence from adult skeletal muscle cDNA. Piétrain and Large White RT-PCR products were sequenced, indicating that the coding sequences [was]were identical in both breeds and with the published sequence. However, a G A transition was found in the leader sequence corresponding to exon 2 in man (Figure 4). We developed a screening test for this single nucleotide polymorphism (SNP) based on the ligation amplification reaction (LAR), allowing us to genotype our pedigree material. Based on these data, *[Igf]/IGF2* was shown to colocalize with the SWC9 microsatellite marker (= 0%), therefore, located at approximately 2 centimorgan from the most likely position of the QTL and well within the 95% support interval for the QTL (figure 1). Subsequent sequence analysis demonstrated that the microsatellite marker SWC9 is actually located within the 3' UTR of the *[Igf]/IGF2* gene. Combined with available comparative mapping data for the PGA and FSH loci, these results suggest the occurrence of an interstitial inversion of a chromosome segment containing *MyoD*, but not *[Igf]/IGF2* which has remained telomeric in both species.

[0076] *[Igf2]/IGF2*, therefore, appeared as a strong positional allele having the observed QTL effect. In man and mouse, *[Igf]/IGF2* is known to be imprinted and to be expressed exclusively from the paternal allele in several tissues<sup>15</sup>. Analysis of skeletal muscle cDNA from pigs heterozygous for the SNP and/or SWC9[,] shows that the same imprinting holds in this tissue in the pig as well (Figure 4). Therefore, if *[Igf]/IGF2* were responsible for the observed effect, and knowing that only the paternal *[Igf2]/IGF2* allele is expressed, one can predict that (i) the paternal allele transmitted by Fl boars (P or LW) would have an effect on the phenotype of F2 offspring, (ii) the maternal allele transmitted by Fl sows (P or LW) would have no effect on phenotype of F2 offspring, and (iii) the likelihood of the data would be superior under a model of a bimodal (1:1) F2 population sorted by inherited paternal allele when compared to a conventional "Mendelian" model of a trimodal (1:2:1)

F2 population. The QTL mapping programmes were adapted in order to allow testing of the corresponding hypotheses.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  as testing for the presence of a Mendelian QTL,  $H_2$  as testing for the presence of a paternally expressed QTL, and  $H_3$  as testing for the presence of a maternally expressed QTL.

[0077] Figure 3 summarizes the obtained results. [Figure]Figures 3a, 3b and 3c respectively show the lodscore curves corresponding to  $\log_{10} (H_2/H_0)$ ,  $\log_{10} (H_3/H_0)$  and  $\log_{10} (H_2/H_1)$ . It can be seen that very significant lodscores are obtained when testing for the presence of a paternally expressed QTL, while there is no evidence at all for the segregation of a QTL when studying the chromosomes transmitted by the sows. Also, the hypothesis of a paternally expressed QTL is significantly more likely ( $\log_{10} (H_2/H_1) > 3$ ) than the hypothesis of a "Mendelian" QTL for all examined traits. The fact that the same tendency is observed for all traits indicates that it is likely the same imprinted gene that is responsible for the effects observed on the different traits. Table 2 reports the ML phenotypic means for the F2 offspring sorted by inherited paternal QTL allele. Note that when performing the analysis under a model of a mendelian QTL, the Piétrain and Large White QTL alleles appeared to behave in an additive fashion, the heterozygous genotype exhibiting a phenotypic mean corresponding exactly to the midpoint between the two [homzygous]homozygous genotypes. This is exactly what one would predict when dealing with an imprinted QTL as [halve]half of the heterozygous offspring are expected to have inherited the P allele from their sire, the other half the LW allele.

[0078] These data, therefore, confirmed our hypothesis of the involvement of an imprinted gene expressed exclusively from the paternal allele. The fact that the identified chromosomal segment coincides precisely with an imprinted domain documented in man and mice strongly implicates the orthologous region in pigs. At least seven imprinted genes mapping to this domain have been documented ([*Igf2*]IGF2, Ins2, H19, Mash2, P57<sup>KIP2</sup>, K<sub>v</sub>LQTL1 and TDAG51) (ref. 15 and Andrew Feinberg, personal communication). Amongst these, only [*Igf2*]IGF2 and Ins2 are paternally expressed. While we cannot exclude that the observed QTL effect is due to an as of yet unidentified imprinted gene in this region, its reported effects on myogenesis *in vitro* and *in vivo*<sup>13</sup> strongly implicate [*Igf2*]IGF2. Particularly the muscular hypertrophy observed in transgenic mice overexpressing [*Igf*]IGF2 from a [muscle specific]muscle-specific promotor are in support of this

hypothesis (Nadia Rosenthal, personal communication). Note that allelic variants of the *INS* VNTR have recently been shown to be associated with size at birth in man<sup>16</sup>, and that the same VNTR has been shown to affect the level of [*Igf2*]*IGF2* expression<sup>17</sup>.

[0079] The observation of the same QTL effect in a Large White x Wild Boar intercross indicates the existence of a series of at least three distinct functional alleles. Moreover, preliminary evidence based on [marker assisted]marker-assisted segregation analysis points towards residual segregation at this locus within the Piétrain population (data not shown). The occurrence of an allelic series might be invaluable in identifying the causal polymorphisms which - based on the [quantitative]quantitative nature of the observed effect - are unlikely to be gross gene alterations but rather subtle regulatory mutations.

[0080] The effects of the identified QTL on muscle mass and fat deposition are truly major, being of the same magnitude of those reported for the *CRC* locus<sup>6,7</sup> though apparently without the associated deleterious effects on meat quality. We estimate that both loci jointly explain close to 50% of the Piétrain versus Large White breed difference for muscularity and leanness. Understanding the parent-of-origin effect characterising this locus will allow for its optimal use in breeding programmes. Indeed, today, half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit the unfavourable Large White allele causing considerable loss.

[0081] The QTL described in this work is the second example of a gene affecting muscle development in livestock species that exhibits a non-mendelian inheritance pattern. Indeed, we have previously shown that the callipyge locus (related to the qualitative trait wherein muscles are doubled) is characterised by polar overdominance in which only the heterozygous individuals that inherit the CLPG mutation from their sire express the double-muscling phenotype<sup>5</sup>. This demonstrates that parent-of-origin effects affecting genes underlying production traits in livestock might be relatively common.

Example 3:

Generating a reference sequence of *IGF2* and flanking loci in the pig.

[0082] The invention provides an imprinted QTL with a major effect on muscle mass mapping to the IGF2 locus in the pig, and use of the QTL as a tool in [marker assisted]marker-assisted selection. To fine tune this tool for [marker assisted]marker-assisted selection, as well as to further identify a causal mutation, we have further generated a reference sequence encompassing the entire porcine IGF2 sequence as well as that from flanking genes.

[0083] To achieve this, we screened a porcine BAC library with IGF2 probes and identified two BACs. BAC-PIGF2-1 proved to contain the INS and IGF2 genes, while BAC-PIGF2-2 proved to contain the IGF2 and H19 genes. The NotI map as well as the relative position of the two BACs is shown in Figure 5. BAC-PIGF2-1 was shotgun sequenced using standard procedures and automatic sequencers. The resulting sequences were assembled using standard software yielding a total of 115 contigs. The corresponding sequences are reported in figure 6. Similarity searches were performed between the porcine contigs and the orthologous sequences in [human]humans. Significant homologies were detected for 18 contigs and are reported in Figure 7.

[0084] For BAC-PIGF2-2, the 24 Kb NotI fragment not present in BACPIGF2-1 was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers. Resulting sequences were assembled using the Phred-Phrap-Consed programme suit, yielding seven distinct contigs (figure 8). The contig sequences were aligned with the corresponding orthologous human sequences using the compare and dotplot programmes of the GCG suite. Figure 9 [syrnmarizes]summarizes the corresponding results.

Example 4: Identification of DNA sequence polymorphisms in the IGF2 and flanking loci.

[0085] Based on the reference sequence obtained as described in Example 1, we resequenced part of the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals, allowing identification of DNA sequence polymorphisms such as reported in figure 10.

[Legends to the Figures

Fig. 1: Test statistic curves obtained in QTL analyses of chromosome 2 in a Wild Boar/Large White intercross. The graph plots the F ratio testing the hypothesis of a single QTL at a given position along the chromosome for the traits indicated. The marker map with the distances between markers in Kosambi centiMorgan is given on the X-axis. The horizontal lines represent genome-wise significant ( $P < 0.05$ ) and suggestive levels for the trait lean meat in ham; similar significance thresholds were obtained for the other traits.

Figure 2: Piétrain pig with characteristic muscular hypertrophy.

[Figure 3] Figures 3A-3C: Lodscore curves obtained in a Piétrain x Large White intercross for six phenotypes measuring muscle mass and fat deposition on pig chromosome 2. The most likely positions of the [*Igf2*]IGF2 and *MyoD* genes determined by linkage analysis with respect to the microsatellite marker map are shown.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  as testing for the presence of a Mendelian QTL,  $H_2$  as testing for the presence of a paternally expressed QTL, and  $H_3$  as testing for the presence of a maternally expressed QTL. [3a]3A:  $\log_{10}(H_1/H_0)$ , [3b]3B:  $\log_{10}(H_2/H_0)$ , [3c]3C:  $\log_{10}(H_3/H_0)$ .

Figure 4: A. Structure of the human [*Igf2*]IGF2 gene according to ref. 17, with aligned porcine adult liver cDNA sequence as reported in ref. 16. The position of the *nt421(G-A)* transition and *Swc9* microsatellite are shown. B. The corresponding markers were used to demonstrate the monoallelic (paternal) expression of [*Igf2*]IGF2 in skeletal muscle and liver of 10-week old fetuses. PCR amplification of the *nt421(G-A)* polymorphism and *Swc9* microsatellite from genomic DNA clearly shows the heterozygosity of the fetus, while only the paternal allele is detected in liver cDNA (*nt421(G-A)* and *Swc9*) and muscle cDNA (*Swc9*). The absence of RT-PCR product for *nt421(G-A)* [from ]in fetal muscle points towards the absence of mRNA including exon 2 in this tissue. Parental origin of the [foetal]fetal alleles was determined from the genotypes of the sire and dam (data not shown).

Figure 5: A NotI restriction map showing the relative position of BAC-PIGF2-1 (comprising INS and IGF2 genes), and BAC-PIGF2-2 (comprising IGF2 and H19 genes).

Figure 6: Nucleic acid sequences of contig 1 to contig 115 derived from BAC-PIGF2-1 which was shotgun sequenced using standard procedures and automatic sequencers.

Figure 7: Similarity between porcine contigs of figure 6 and orthologous sequences in human.

Figure 8: Nucleic acid sequences of contig 1 to contig 7 derived from BAC-PIGF2-2, (the 24 Kb NotI fragment not present in BAC-PIGF2-1) which was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers.

Figure 9: Similarity between porcine contigs of figure 8 and orthologous sequences in human.

Figure 10: DNA sequence polymorphisms in the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals.]

## REFERENCES

Literature cited with example 1

1. Andersson, L. *et al.* Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science* 263, 1771-1774 (1994).
2. Knott, S.A. *et al.* Multiple marker mapping of quantitative trait loci in a cross between outbred wild boar and Large White pigs. *Genetics* 149, 1069-1080 (1998).
3. Edfors-Lilja, I. *et al.* Mapping quantitative trait loci for immune capacity in the pig. *Journal of Immunology* 161, 829-835 (1998).
4. Andersson-Eklund, L. *et al.* Mapping quantitative trait loci for carcass and meat quality traits in a wild boar x Large White intercross. *Journal of Animal Science* 76, 694-700 (1998).
5. Fronicke, L., Chowdhary, B.P., Scherthan, H. & Gustavsson, I. A comparative map of the porcine and human genomes demonstrates ZOO-FISH and gene mapping-based chromosomal homologies. *Mamm Genome* 7, 285-90 (1996).
6. Alexander, L.J. *et al.* Physical assignments of 68 porcine cosmids and lambda clones containing microsatellites. *Mammalian Genome* 7, 368-372 (1996).



7. Rohrer, G.A. et al. A comprehensive map of the porcine genome. *Genome Research* 6, 371-391 (1996).
8. Marklund, L. et al. A comprehensive linkage map of the pig based on a wild pig-Large White intercross. *Anim Genet* 27, 255-69 (1996).
9. Marklund, L., Nyström, P.E., Stern, S., Andersson-Eklund, L. & Andersson, L. Quantitative trait loci for fatness and growth on pig chromosome 4. *Heredity* In press (1998).
10. Ohlsson, R., Hedborg, F., Holmgren, L., Walsh, C. & [Ekstrom]Ekström, T.J. Overlapping patterns of IGF2 and H19 expression during human development: biallelic IGF2 expression correlates with a lack of H19 expression. *Development* 120, 361-368 (1994).
11. Ekström, T.J., Cui, H., Li, X. & Ohlsson, R. Promoter-specific IGF2 imprinting status and its plasticity during human liver development. *Development* 121, 309-316 (1995).
12. Hemberger, M. et al. H19 and [Igf2]IGF2 are expressed and differentially imprinted in neuroectoderm-derived cells in the mouse brain. *Dev. Genes Evol.* 208, 393-402 (1998).
13. Dunger, D.B. et al. Association of the INS VNTR with size at birth. *Nature Genetics* 19, 98-100 (1998).

14. DeChiara, T.M., Robertson, E.J. & Efstratiadis, A. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* 64, 849—859 (1991).
15. Sun, F.L., Dean, W.L., Kelsey, G., Allen, N.D. & Reik, W. Transactivation of [Igf2]IGF2 in a mouse model of Beckwith-Wiedemann syndrome. *Nature* 389, 809-815 (1997).
16. Davies, J.L. *et al.* A genome-wide search for human type 1 diabetes susceptibility genes. *Nature* 371, 130-136 (1994).
17. O'Dell, S.D. *et al.* ApaI polymorphism in insulin-like growth factor II (IGF2) gene and weight in middle-aged males. *International Journal of Obesity* 21, 822—825 (1997).
18. Falconer, D.S. & Mackay, T.F.C. *Introduction to Quantitative Genetics*, (Longman, England, 1996).
19. Hill, W.G. Rates of change in quantitative traits from fixation of new mutations. *Proc Natl Acad Sci U S A* 79, 142-145 (1982).
20. Marklund, S. *et al.* Molecular basis for the dominant white phenotype in the domestic pig. *Genome Research* 8, 826-833 (1998).
21. Kijas, J.M.H. *et al.* Melanocortin receptor 1 (*MC1R*) mutations and coat color in the pig. *Genetics* In press(1998).

22. Beechey, C.V. personal communication (1998).
23. Paquette, J., Giannoukakis, N., Polychronakos, C., Vafiadis, P. & Deal, C. The *INS* 5' variable number of tandem repeats is associated with IGF2 expression in humans. *Journal of Biological Chemistry* 273, 14158-14164 (1998).
24. Sambrook, J., Fritsch, E.F. & Maniatis, T. *Molecular cloning: A laboratory manual.*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989).
25. Chowdhary, B.P., de la Sena, C., Harbitz, I., Eriksson, L. & Gustavsson, I. FISH on metaphase and interphase chromosomes demonstrates the physical order of the genes for GPI, CRC, and LIPE in pigs. *Cytogenetics Cell Genetics* 71, 175-178 (1995).
26. Green, P., Falls, K. & Crook, S. *Documentation for CRI-MAP, version 2.4.*, (Washington University School of Medicine, St. [Louise]Louis, MO, 1990).
27. Haley, C.S., Knott, S.A. & Elsen, J.M. Mapping quantitative trait loci in crosses between outbred lines using least squares. *Genetics* 136, 1195-1207 (1994).
28. Churchill, G.A. & Doerge, R.W. Empirical threshold values for quantitative trait mapping. *Genetics* 138, 963-971 (1994).
29. Anonymous. *SAS version 6.10*, (SAS Institute Inc., Cary, NC., 1990).

References used with example 2:

1. Fuji, J.; Otsu, K.; Zorzato, F.; Deleon, S.; Khanna, V.K.; Weiler, J.E. O'Brien, P.J.; MacLennan, D.H. (1991). Identification of a mutation in the porcine ryanodine receptor associated with malignant hyperthermia. *Science* 253: 448-451.
2. MacLennan, D.H. & Phillips, M.S. (1993). Malignant hyperthermia. *Science* 256:789-794.
3. Grobet, L.; Royo Martin, L.J.; Poncelet, D.; Pirottin, D.; Brouwers, B.; Riquet, J.; Schoeberlein, A.; Dunner, S.; Ménéssier, F.; Massabanda, J.; Fries, R.; Hanset, R.; Georges, M. (1997) A deletion in the myostatin gene causes double muscling in cattle. *Nature Genetics* 17:71-74.
4. Andersson, L.; Haley, C.S.; Ellegren, H.; Knott, S.A.; Johansson, M.; Andersson, K.; Andersson-Eklund, L.; Edfors-Lilja, I.; Fredholm, M.; Hansson, I.; Hakansson, J.; Lundström, K. (1994). Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science* 263:1771-1774.
5. Cockett, N.; Jackson, S.; Shaw, T.; Farnir, F.; Berghmans, S.; Snowden, G.; Nielsen, D.; Georges, M. (1996). Polar overdominance at the ovine callipyge locus. *Science* 273:236-238.
6. Hanset, R.; Dasnois, C.; Scalais, S.; Michaux, C.; Grobet, L. (1995). Genotypes at the locus for halothane sensitivity and performance in a Piétrain x Large White F2. *Genet. Sel. Evol.* 27: 63-76.

7. Hanset, R.; Dasnois, C.; Scalais, S.; Michaux, C.; Grobet, L. (1995). Introgression into the Piétrain genome of the normal allele at the locus for halothane sensitivity. *Genet. Sel. Evol.* 27: 77-88.
8. Olivier, L.; Lauvergne, J.J. (1967). A study of the inheritance of the muscular hypertrophy of the Piétrain pig: preliminary results. *Annales de Médecine Vétérinaire* 111: 104-109.
9. Rettenberger, G.; Klett, C.; Zechner, U.; Kunz, J.; Vogel, W.; Hameister, H. (1995). Visualisation of the conservation of synteny between humans and pigs by heterologous chromosome painting. *Genomics* 26: 372-378.
10. Goureau, A.; Yerle, M.; Schmitz, A.; Riquet, J.; Milan, D.; Pinton, P.; Frelat, G.; Gellin, J. (1996). Human and porcine correspondence of chromosome segments using bidirectional chromosome painting. *Genomics* 36:252-262.
11. Yun, K.; Wold, B. (1996). Skeletal muscle determination and differentiation: story of a core regulatory network and its context. *Current Opinion in Cell Biology* 8:877-889.
12. Knoll, A.; Nebola, M.; Dvorak, J.; Cepica, S. (1997). Detection of a DdeI PCR RFLP within intron 1 of the porcine MYOD1(MYF3) locus. *Animal Genetics* 28, 308-322.
13. Florini, J.R.; Ewton, D.Z.; McWade, F.J. (1995). IGFs, muscle growth, and myogenesis. *Diabetes Review* 3:73-92.

14. Catchpole, I.R.; Engström, W. (1990). Nucleotide sequence of a porcine insulin-like growth factor II cDNA. *Nucleic Acids Research* 18(21):6430.
  
15. Feil, R.; Moore, T.F.; Oswald, J.; Walter, J.; Sun, F.; Reik, W. (1997). The imprinted insulin like growth factor 2 gene. Pp70 In *Genomic Imprinting*. Eds. Reik & Surani. IRL Press at Oxford University Press..
  
16. Dunger, D.B.; Ong, K.K.L.; Huxtable, S.J.; Sherriff, A.; Woods, K.A.; Ahmed, M.L.; Golding, J.; Pembrey, M.E.; Ring, S.; the AISPAC study team, Bennett, S.T.; Todd, J.A. (1998). Association of the INS VNTR with size at birth. *Nature Genetics* 19: 98-100.
  
17. Paquette, J., Giannoukakis, N., Polychronakos, C., Vafiadis, P., Deal, C. (1998). The INS 5' variable number of tandem repeats is associated with IGF2 expression in humans. *J. Biol Chem* 273 (23):14158-14164.
  
18. Andersson-Eklund, L.; Marklund, L.; Lundström, K.; Haley, C.S.; Andersson, K.; Hansson, I.; Moller, M.; Andersson, L. (1998). Mapping Quantitative Trait Loci for carcass and meat quality traits in a Wild Boar x Large White intercross. *J. Anim. Sci.* 76:694-700.
  
19. Rohrer, G.A.; Alexander, L.J.; Hu, Z.; Keele, J.W.; Smith, T.P.; Beattie, C.W. (1996). A comprehensive map of the porcine genome. *Genome Research*, in the press.

20. Georges, M.; Nielsen, D.; Mackinnon, M.; Mishra, A.; Okimoto, R.; Pasquino, A.T.; Sargeant, L.S.; Sorensen, A.; Steele, M.R.; Zhao, X.; Womack, J.E.; Hoeschele, I. (1995). Mapping quantitative trait loci controlling milk production by exploiting progeny testing. *Genetics* 139: 907-920.
  
21. Baron, H.; Fung, S.; Aydin, A.; Bahring, S.; Luft, F.C.; Schuster, H. (1996). Oligonucleotide ligation assay (OLA) for the diagnosis of familial hypercholesterolemia. *Nat. Biotechnol.* 14(10):1279-1282.
  
22. Lander, E.; Green, P. (1987) Construction of multilocus genetic linkage maps in humans. *Proceedings of National Academy of Science (USA)* 84: 2363-2367.
  
23. Lalouel, J.M. (1983). Optimization of functions. *Contrib. Epidemiol. Biostat.* 4:235-259.
  
24. Lander, E.S. & Botstein, D. (1989). Mapping mendelian [factots]factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185-199.
  
25. Spelman[ RL,], R.L.; Coppieters, W[,]; Karim, L[,]; van Arendonk[ JAM,], J.A.M.; Bovenhuis, H. (1996). Quantitative trait loci analysis for five milk production traits on chromosome six in the dutch Holstein-Friesian population. *Genetics* 144:1799-1808.

26. Chirgwin, J.M.; Przybyla, A.E.; MacDonald, R.J.; Rutter, W.J. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18:5294-5299.



Table 1 Summary of QTL analysis for pig chromosome 2 in a Wild Boar/Large White intercross<sup>1</sup>

Trait	F ratio <sup>2</sup> <i>QTL</i>	<i>Imprinting</i>	Map position <sup>3</sup>	Percent of F <sub>2</sub> variance <sup>4</sup>	Least squares means <sup>5</sup>		
					$W^P/W^M$	$W^P/L^M$	$L^P/W^M$
<i>L<sup>P</sup>/L<sup>M</sup></i>							
<u>Body composition traits</u>							
Lean meat in ham, %	24.4***	19.1***	0	30.6	n=62 63.6 <sup>a</sup>	n=43 64.2 <sup>a</sup>	n=30 67.3 <sup>b</sup>
Lean meat mass in ham, kg	18.1***	16.8***	1	24.3	4.69 <sup>a</sup>	4.72 <sup>a</sup>	5.02 <sup>b</sup>
Lean meat + bone in back, %	12.2**	9.6**	0	17.4	66.3 <sup>a</sup>	66.7 <sup>a</sup>	70.8 <sup>b</sup>
Longissimus muscle area, cm <sup>2</sup>	10.3**	4.8*	1	15.4	31.9 <sup>a</sup>	33.0 <sup>a</sup>	35.2 <sup>b</sup>
<u>Fatness traits</u>							
Average [back fat]/backfat depth, mm	7.1*	8.7**	0	10.4	27.2 <sup>a</sup>	27.7 <sup>a</sup>	24.7 <sup>b</sup>
<u>Weight of internal organs</u>							
Heart, gram	9.7**	11.4***	0	14.4	226 <sup>a</sup>	225 <sup>a</sup>	244 <sup>b</sup>
<u>Meat quality traits</u>							
Reflectance value, EEL	5.7	6.1*	1	8.1	18.6 <sup>a</sup>	18.4 <sup>a</sup>	19.7 <sup>a</sup>

\*P&lt;0.05; \*\*P&lt;0.01; \*\*\*P&lt;0.001

**Table 1, continued**

<sup>1</sup>Only the traits for which the QTL peak was in the IGF2 region (0-10 cM) and the test statistic [reached]reaching the nominal significance threshold of  $F=3.9$  are included.

<sup>2</sup>"*QTL*" is the test statistic for the presence of a QTL under a genetic model with additive, dominance, and imprinting effects (3 d.f.) while "Imprinting" is the test statistic for the presence of an imprinting effect (1 d.f.), both obtained at the position of the QTL peak. Genome-wise significance thresholds, estimated by permutation, were used for the QTL test while nominal significance thresholds were used for the Imprinting test.

<sup>3</sup>1n cM from the distal end of 2p; IGF2 is located at 0.3 cM.

<sup>4</sup>The reduction in the residual variance of the  $F_2$  population effected by inclusion of an imprinted QTL at the given position.

<sup>5</sup>Means and standard errors estimated at the IGF2 locus by classifying the genotypes according to the population and parent of origin of each allele. *W* and *L* represent alleles derived from the Wild Boar and Large White founders, respectively; [superscript]superscripts *P* and *M* represent a paternal and maternal origin, respectively. Figures with different letters (superscript a or b) are significantly different at least at the 5% level, and most of them are different at the 1% or 0.1% level.

Table 2 Maximum likelihood phenotypic means for the different F2 genotypes estimated under (i) a model of a mendelian QTL, and (ii) a model assuming an imprinted QTL.

Traits	Mendelian QTL				Imprinted QTL		
	$\mu_{LW/LW}$	$\mu_{LW/P}$	$\mu_{P/P}$	R	$\mu_{PAT/LW}$	$\mu_{PAT/P}$	R
BFT (cm)	2.98	2.84	2.64	0.27	2.94	2.70	0.27
% ham	21.10	21.56	22.15	0.83	21.23	21.95	0.83
% loin	24.96	25.53	26.46	0.91	25.12	26.14	0.93
% lean cuts	65.02	65.96	67.60	1.65	65.23	67.05	1.67
% backfat	6.56	6.02	5.33	0.85	6.43	5.56	0.85
% fat cuts	28.92	27.68	26.66	1.46	28.54	26.99	1.49

PATENT  
4951US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

**In re Application of:**

ANDERSSON et al.

**Serial No.:** 09/868,732

**Filed:** June 15, 2001

**For:** SELECTING ANIMALS FOR  
PARENTALLY IMPRINTED TRAITS

**Examiner:** To be assigned

**Group Art Unit:** To be assigned

**Attorney Docket No.:** 4951US

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number: EL740548284 US

Date of Deposit with USPS: November 1, 2001

Person making Deposit: Daniel Thatcher

AMENDMENT

Box Non-Fee Amendment  
Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination of the above-referenced patent application on the merits, entry of the amendments as set forth herein is respectfully solicited.

Pursuant to 37 C.F.R. §§ 1.121(b)(1)(i), please delete paragraphs [0019], [0021], [0023], [0053], [0054], and [0069] of the substitute specification mailed by the applicants on September 17, 2001, and replace the same with the like-numbered replacement paragraphs [0019], [0021], [0023], [0053], [0054], and [0069] set forth herein. In compliance with 37 C.F.R. §§ 1.121(b)(1)(ii), the above-referenced replacement paragraphs are submitted in clean form below. A version of the replacement paragraphs with markings to show changes made relative to the previous version of such

paragraphs is appended hereto under the title “VERSION WITH MARKINGS TO SHOW CHANGES MADE,” pursuant to 37 C.F.R. §§ 1.121(b)(1)(iii).

IN THE SPECIFICATION:

[0019] Figure 6: Nucleic acid sequences of contig 1 (SEQ ID NO:10), contig 2 (SEQ ID NO:11), contig 3 (SEQ ID NO:12), contig 4 (SEQ ID NO:13), contig 5 (SEQ ID NO:14), contig 6 (SEQ ID NO:15), contig 7 (SEQ ID NO:16), contig 8 (SEQ ID NO:17), contig 9 (SEQ ID NO:18), contig 10 (SEQ ID NO:19), contig 19 (SEQ ID NO:20), contig 20 (SEQ ID NO:21), contig 21 (SEQ ID NO:22), contig 22 (SEQ ID NO:23), contig 23 (SEQ ID NO:24), contig 24 (SEQ ID NO:25), contig 25 (SEQ ID NO:26), contig 26 (SEQ ID NO:27), contig 27 (SEQ ID NO:28), contig 28 (SEQ ID NO:29), contig 29 (SEQ ID NO:30), contig 30 (SEQ ID NO:31), contig 31 (SEQ ID NO:32), contig 32 (SEQ ID NO:33), contig 33 (SEQ ID NO:34), contig 34 (SEQ ID NO:35), contig 35 (SEQ ID NO:36), contig 36 (SEQ ID NO:37), contig 37 (SEQ ID NO:38), contig 38 (SEQ ID NO:39), contig 39 (SEQ ID NO:40), contig 40 (SEQ ID NO:41), contig 41 (SEQ ID NO:42), contig 42 (SEQ ID NO:43), contig 43 (SEQ ID NO:44), contig 44 (SEQ ID NO:45), contig 45 (SEQ ID NO:46), contig 46 (SEQ ID NO:47), contig 47 (SEQ ID NO:48), contig 48 (SEQ ID NO:49), contig 49 (SEQ ID NO:50), contig 50 (SEQ ID NO:51), contig 51 (SEQ ID NO:52), contig 52 (SEQ ID NO:53), contig 53 (SEQ ID NO:54), contig 54 (SEQ ID NO:55), contig 55 (SEQ ID NO:56), contig 56 (SEQ ID NO:57), contig 57 (SEQ ID NO:58), contig 58 (SEQ ID NO:59), contig 59 (SEQ ID NO:60), contig 60 (SEQ ID NO:61), contig 61 (SEQ ID NO:62), contig 62 (SEQ ID NO:63), contig 63 (SEQ ID NO:64), contig 64 (SEQ ID NO:65), contig 65 (SEQ ID NO:66), contig 66 (SEQ ID NO:67), contig 67 (SEQ ID NO:68), contig 68 (SEQ ID NO:69), contig 69 (SEQ ID NO:70), contig 70 (SEQ ID NO:71), contig 71 (SEQ ID NO:72), contig 72 (SEQ ID NO:73), contig 73 (SEQ ID NO:74), contig 74 (SEQ ID NO:75), contig 75 (SEQ ID NO:76), contig 76 (SEQ ID NO:77), contig 77 (SEQ ID NO:78), contig 78 (SEQ ID NO:79), contig 79 (SEQ ID NO:80), contig 80 (SEQ ID NO:81), contig 81 (SEQ ID NO:82), contig 82 (SEQ ID NO:83), contig 83 (SEQ ID NO:84), contig 84 (SEQ ID NO:85), contig 85 (SEQ ID NO:86), contig 86 (SEQ ID NO:87), contig 87 (SEQ ID NO:88), contig 88 (SEQ ID NO:89), contig 89 (SEQ ID NO:90), contig 90 (SEQ ID NO:91), contig 91 (SEQ

ID NO:92), contig 92 (SEQ ID NO:93), contig 93 (SEQ ID NO:94), contig 94 (SEQ ID NO:95), contig 95 (SEQ ID NO:96), contig 96 (SEQ ID NO:97), contig 97, (SEQ ID NO:98), contig 98 (SEQ ID NO:99), contig 99 (SEQ ID NO:100), contig 100 (SEQ ID NO:101), contig 101 (SEQ ID NO:102), contig 102 (SEQ ID NO:103), contig 103 (SEQ ID NO:104), contig 104 (SEQ ID NO:105), contig 105 (SEQ ID NO:106), contig 106 (SEQ ID NO:107), contig 107 (SEQ ID NO:108), contig 108 (SEQ ID NO:109), contig 109 (SEQ ID NO:110), contig 110 (SEQ ID NO:111), contig 111 (SEQ ID NO:112), contig 112 (SEQ ID NO:113), contig 113 (SEQ ID NO:114), contig 114 (SEQ ID NO:115), and contig 115 (SEQ ID NO:116) derived from BAC-PIGF2-1, which was shotgun sequenced using standard procedures and automatic sequencers.

**[0021]** Figure 8: Nucleic acid sequences of contig 1 (SEQ ID NO:117), contig 2 (SEQ ID NO:118), contig 3 (SEQ ID NO:119), contig 4 (SEQ ID NO:120), contig 5 (SEQ ID NO:121), contig 6 (SEQ ID NO:122), and contig 7 (SEQ ID NO:123) derived from BAC-PIGF2-2, (the 24 Kb NotI fragment not present in BAC-PIGF2-1), which was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers.

**[0023]** Figure 10: DNA sequence polymorphisms in the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals. Polymorphisms 1 through 4 occur in contig 3 (SEQ ID NO:12), polymorphisms 5 through 23 occur in contig 4 (SEQ ID NO: 13), polymorphisms 24 through 28 occur in contig 10 (SEQ ID NO:19), polymorphism 29 occurs in contig 57 (SEQ ID NO:58), polymorphism 20 occurs in contig 95 (SEQ ID NO:96), and polymorphism 31 occurs in contig 105 (SEQ ID NO:106).

**[0053]** Isolation of an IGF2 BAC clone and fluorescent *in situ* hybridisation (FISH). IGF2 primers (F:5'- GGCAAGTTCTTCCGCTAATGA-3' (SEQ ID NO:1) and R:5' - GCACCGCAGAATTACGACAA-3' (SEQ ID NO:2)) for PCR amplification of a part of the last exon and 3'UTR were designed on the basis of a porcine IGF2 cDNA sequence (GenBank X56094). The primers were used to screen a porcine BAC library and the clone 253G10 was isolated. Crude BAC DNA was prepared as described<sup>24</sup>. The BAC DNA was linearized with *EcoRV* and purified

with QIAEXII (QIAGEN GmbH, Germany). The clone was labeled with biotin-14-dATP using the GIBCO-BRL Bionick labeling system (BRL18246-015). Porcine metaphase chromosomes were obtained from pokeweed (Seromed) stimulated lymphocytes using standard techniques. The slides were aged for two days at room temperature and then kept at -20°C until use. FISH analysis was carried out as previously described<sup>25</sup>. The final concentration of the probe in the hybridisation mix was 10 ng/ $\mu$ l. Repetitive sequences were suppressed with standard concentrations of porcine genomic DNA. After post-hybridisation washing, the biotinylated probe was detected with two layers of avidin-FITC (Vector A-2011). The chromosomes were counterstained with 0.3 mg/ml DAPI (4, 6-Diamino-2-phenylindole; Sigma D9542), which produced a G-banding like pattern. No posthybridisation banding was needed, since chromosome 2 is easily recognized without banding. A total of 20 metaphase spreads were examined under an Olympus BX-60 fluorescence microscope connected to an IMAC-CCD S30 video camera and equipped with an ISIS 1.65 (Metasystems) software.

**[0054]** About two  $\mu$ g of linearized and purified BAC DNA was used for direct sequencing with 20 pmoles of primers and BigDye Terminator chemistry (Perkin Elmer, USA). DNA sequencing was done from the 3' end of the last exon towards the 3' end of the UTR until a microsatellite was detected. A primer set (F:5'-GTTTCTCCTGTACCCACACGCATCCC-3' (SEQ ID NO:3) and R: 5'-Fluorescein-CTACAAGCTGGGCTCAGGG-3' (SEQ ID NO:4)) was designed for the amplification of the IGF2 microsatellite which is about 250 bp long and located approximately 800 bp downstream from the stop codon. The microsatellite was PCR amplified using fluorescently labeled primers and the genotyping was carried out using an ABI377 sequencer and the GeneScan/Genotyper softwares (Perkin Elmer, USA). Two-point and multipoint linkage analyses were done with the Cri-Map software<sup>26</sup>.

**[0069]** *Marker genotyping:* Primer pairs utilised for PCR amplification of microsatellite markers are as described<sup>19</sup>. Marker genotyping was performed as previously described<sup>20</sup>. Genotypes at the *CRC* and *MyoD* loci were determined using conventional methods as described<sup>1,12</sup>. The LAR test for the IGF2 SNP was developed according to Baron et al.<sup>21</sup> using a primer pair for PCR

amplification (5'-CCCCTGAACTTGAGGACGAGCAGCC-3'(SEQ ID NO:5); 5'-ATCGCTGTGGGCTGGGTGGGCTGCC-3')(SEQ ID NO:6) and a set of three primers for the LAR step (5'-FAM-CGCCCCAGCTGCCCCCAG-3'(SEQ ID NO:7); 5' -HEX-CGCCCCAGCTGCCCCCAA-3'(SEQ ID NO:8); 5' -CCTGAGCTGCAGCAGGCCAG-3' (SEQ ID NO:9)).



**REMARKS**

No new matter has been added. The Applicants again request entry of the amendments as set forth herein prior to examination of the application on the merits.

Respectfully submitted,



Shawn G. Hansen  
Registration No. 42,627  
Attorney for Applicants  
TRASKBRITT, PC  
P. O. Box 2550  
Salt Lake City, Utah 84110-2550  
Telephone: (801) 532-1922

Date: November 1, 2001

## VERSION WITH MARKINGS TO SHOW CHANGES MADE

### IN THE SPECIFICATION:

[0019] Figure 6: Nucleic acid sequences of contig 1 (SEQ ID NO:10), contig 2 (SEQ ID NO:11), contig 3 (SEQ ID NO:12), contig 4 (SEQ ID NO:13), contig 5 (SEQ ID NO:14), contig 6 (SEQ ID NO:15), contig 7 (SEQ ID NO:16), contig 8 (SEQ ID NO:17), contig 9 (SEQ ID NO:18), contig 10 (SEQ ID NO:19), contig 19 (SEQ ID NO:20), contig 20 (SEQ ID NO:21), contig 21 (SEQ ID NO:22), contig 22 (SEQ ID NO:23), contig 23 (SEQ ID NO:24), contig 24 (SEQ ID NO:25), contig 25 (SEQ ID NO:26), contig 26 (SEQ ID NO:27), contig 27 (SEQ ID NO:28), contig 28 (SEQ ID NO:29), contig 29 (SEQ ID NO:30), contig 30 (SEQ ID NO:31), contig 31 (SEQ ID NO:32), contig 32 (SEQ ID NO:33), contig 33 (SEQ ID NO:34), contig 34 (SEQ ID NO:35), contig 35 (SEQ ID NO:36), contig 36 (SEQ ID NO:37), contig 37 (SEQ ID NO:38), contig 38 (SEQ ID NO:39), contig 39 (SEQ ID NO:40), contig 40 (SEQ ID NO:41), contig 41 (SEQ ID NO:42), contig 42 (SEQ ID NO:43), contig 43 (SEQ ID NO:44), contig 44 (SEQ ID NO:45), contig 45 (SEQ ID NO:46), contig 46 (SEQ ID NO:47), contig 47 (SEQ ID NO:48), contig 48 (SEQ ID NO:49), contig 49 (SEQ ID NO:50), contig 50 (SEQ ID NO:51), contig 51 (SEQ ID NO:52), contig 52 (SEQ ID NO:53), contig 53 (SEQ ID NO:54), contig 54 (SEQ ID NO:55), contig 55 (SEQ ID NO:56), contig 56 (SEQ ID NO:57), contig 57 (SEQ ID NO:58), contig 58 (SEQ ID NO:59), contig 59 (SEQ ID NO:60), contig 60 (SEQ ID NO:61), contig 61 (SEQ ID NO:62), contig 62 (SEQ ID NO:63), contig 63 (SEQ ID NO:64), contig 64 (SEQ ID NO:65), contig 65 (SEQ ID NO:66), contig 66 (SEQ ID NO:67), contig 67 (SEQ ID NO:68), contig 68 (SEQ ID NO:69), contig 69 (SEQ ID NO:70), contig 70 (SEQ ID NO:71), contig 71 (SEQ ID NO:72), contig 72 (SEQ ID NO:73), contig 73 (SEQ ID NO:74), contig 74 (SEQ ID NO:75), contig 75 (SEQ ID NO:76), contig 76 (SEQ ID NO:77), contig 77 (SEQ ID NO:78), contig 78 (SEQ ID NO:79), contig 79 (SEQ ID NO:80), contig 80 (SEQ ID NO:81), contig 81 (SEQ ID NO:82), contig 82 (SEQ ID NO:83), contig 83 (SEQ ID NO:84), contig 84 (SEQ ID NO:85), contig 85 (SEQ ID NO:86), contig 86 (SEQ ID NO:87), contig 87 (SEQ ID NO:88), contig 88 (SEQ ID NO:89), contig 89 (SEQ ID NO:90), contig 90 (SEQ ID NO:91),

contig 91 (SEQ ID NO:92), contig 92 (SEQ ID NO:93), contig 93 (SEQ ID NO:94), contig 94 (SEQ ID NO:95), contig 95 (SEQ ID NO:96), contig 96 (SEQ ID NO:97), contig 97, (SEQ ID NO:98), contig 98 (SEQ ID NO:99), contig 99 (SEQ ID NO:100), contig 100 (SEQ ID NO:101), contig 101 (SEQ ID NO:102), contig 102 (SEQ ID NO:103), contig 103 (SEQ ID NO:104), contig 104 (SEQ ID NO:105), contig 105 (SEQ ID NO:106), contig 106 (SEQ ID NO:107), contig 107 (SEQ ID NO:108), contig 108 (SEQ ID NO:109), contig 109 (SEQ ID NO:110), contig 110 (SEQ ID NO:111), contig 111 (SEQ ID NO:112), contig 112 (SEQ ID NO:113), contig 113 (SEQ ID NO:114), contig 114 (SEQ ID NO:115), and [to ]contig 115 (SEQ ID NO:116) derived from BAC-PIGF2-1, which was shotgun sequenced using standard procedures and automatic sequencers.

**[0021]** Figure 8: Nucleic acid sequences of contig 1 (SEQ ID NO:117), contig 2 (SEQ ID NO:118), contig 3 (SEQ ID NO:119), contig 4 (SEQ ID NO:120), contig 5 (SEQ ID NO:121), contig 6 (SEQ ID NO:122), and [to ]contig 7 (SEQ ID NO:123) derived from BAC-PIGF2-2, (the 24 Kb NotI fragment not present in BAC-PIGF2-1), which was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers.

**[0023]** Figure 10: DNA sequence polymorphisms in the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals. Polymorphisms 1 through 4 occur in contig 3 (SEQ ID NO:12), polymorphisms 5 through 23 occur in contig 4 (SEQ ID NO: 13), polymorphisms 24 through 28 occur in contig 10 (SEQ ID NO:19), polymorphism 29 occurs in contig 57 (SEQ ID NO:58), polymorphism 20 occurs in contig 95 (SEQ ID NO:96), and polymorphism 31 occurs in contig 105 (SEQ ID NO:106).

**[0053]** Isolation of an IGF2 BAC clone and fluorescent *in situ* hybridisation (FISH). IGF2 primers (F:5'- GGCAAGTTCTTCCGCTAATGA-3' [(SEQ. ID. NO: \_\_\_\_)](SEQ ID NO:1) and R:5'-GCACCGCAGAATTACGACAA-3' [(SEQ. ID. NO: \_\_\_\_)](SEQ ID NO:2)) for PCR amplification of a part of the last exon and 3'UTR were designed on the basis of a porcine IGF2 cDNA sequence (GenBank X56094). The primers were used to screen a porcine

BAC library and the clone 253G10 was isolated. Crude BAC DNA was prepared as described<sup>24</sup>. The BAC DNA was linearized with *EcoRV* and purified with QIAEXII (QIAGEN GmbH, Germany). The clone was labeled with biotin-14-dATP using the GIBCO-BRL Bionick labeling system (BRL18246-015). Porcine metaphase chromosomes were obtained from pokeweed (Seromed) stimulated lymphocytes using standard techniques. The slides were aged for two days at room temperature and then kept at -20°C until use. FISH analysis was carried out as previously described<sup>25</sup>. The final concentration of the probe in the hybridisation mix was 10 ng/μl. Repetitive sequences were suppressed with standard concentrations of porcine genomic DNA. After post-hybridisation washing, the biotinylated probe was detected with two layers of avidin-FITC (Vector A-2011). The chromosomes were counterstained with 0.3 mg/ml DAPI (4, 6-Diamino-2-phenylindole; Sigma D9542), which produced a G-banding like pattern. No posthybridisation banding was needed, since chromosome 2 is easily recognized without banding. A total of 20 metaphase spreads were examined under an Olympus BX-60 fluorescence microscope connected to an IMAC-CCD S30 video camera and equipped with an ISIS 1.65 (Metasystems) software.

**[0054]** About two μg of linearized and purified BAC DNA was used for direct sequencing with 20 pmoles of primers and BigDye Terminator chemistry (Perkin Elmer, USA). DNA sequencing was done from the 3' end of the last exon towards the 3' end of the UTR until a microsatellite was detected. A primer set (F: 5'-GTTTCTCCTGTACCCACACGCATCCC-3' [(SEQ. ID. NO: \_\_\_\_)](SEQ ID NO:3) and R: 5'-Fluorescein-CTACAAGCTGGGCTCAGGG-3') [(SEQ. ID. NO: \_\_\_\_)](SEQ ID NO:4) was designed for the amplification of the IGF2 microsatellite which is about 250 bp long and located approximately 800 bp downstream from the stop codon. The microsatellite was PCR amplified using fluorescently labeled primers and the genotyping was carried out using an ABI377 sequencer and the GeneScan/Genotyper softwares (Perkin Elmer, USA). Two-point and multipoint linkage analyses were done with the Cri-Map software<sup>26</sup>.

**[0069]** *Marker genotyping:* Primer pairs utilised for PCR amplification of microsatellite

markers are as described<sup>19</sup>. Marker genotyping was performed as previously described<sup>20</sup>. Genotypes at the *CRC* and *MyoD* loci were determined using conventional methods as described<sup>1, 12</sup>. The LAR test for the IGF2 SNP was developed according to Baron et al.<sup>21</sup> using a primer pair for PCR amplification (5'-CCCCTGAACTTGAGGACGAGCAGCC-3' [(SEQ. ID. NO. \_\_\_\_)](SEQ ID NO:5); 5'-ATCGCTGTGGGCTGGGTGGGCTGCC-3') [(SEQ. ID. NO. \_\_\_\_)](SEQ ID NO:6) and a set of three primers for the LAR step (5'-FAM-CGCCCCAGCTGCCCCCAG-3' [(SEQ. ID. NO. \_\_\_\_)](SEQ ID NO:7); 5'-HEX-CGCCCCAGCTGCCCCCAA-3' [(SEQ. ID. NO. \_\_\_\_)](SEQ ID NO:8); 5'-CCTGAGCTGCAGCAGGCCAG-3') [(SEQ. ID. NO. \_\_\_\_)](SEQ ID NO:9).

Title: Selecting animals for parentally imprinted traits.

5

The invention relates to methods to select breeding animals or animals destined for slaughter for having desired genotypic or potential phenotypic properties, in particular related to muscle mass and/or fat deposition. Breeding schemes for domestic animals have so far focused on farm performance traits and carcass quality. This has resulted in substantial improvements in traits like reproductive success, milk production, lean/fat ratio, prolificacy, growth rate and feed efficiency. Relatively simple performance test data have been the basis for these improvements, and selected traits were assumed to be influenced by a large number of genes, each of small effect (the infinitesimal gene model). There are now some important changes occurring in this area. First, the breeding goal of some breeding organisations has begun to include meat quality attributes in addition to the "traditional" production traits. Secondly, evidence is accumulating that current and new breeding goal traits may involve relatively large effects (known as major genes), as opposed to the infinitesimal model that has been relied on so far.

Modern DNA-technologies provide the opportunity to exploit these major genes, and this approach is a very promising route for the improvement of meat quality, especially since direct meat quality assessment is not viable for potential breeding animals. Also for other traits such as lean/fat ratio, growth rate and feed efficiency, modern DNA technology can be very effective. Also these traits are not always easy to measure in the living animal.

The evidence for several of the major genes originally obtained using segregation analysis, i.e. without any DNA marker information. Afterwards molecular studies were performed to detect the location of these

genes on the genetic map. In practice, and except for alleles of very large effect, DNA studies are required to dissect the genetic nature of most traits of economic importance. DNA markers can be used to localise genes or alleles responsible for qualitative traits like coat colour, and they can also be used to detect genes or alleles with substantial effects on quantitative traits like growth rate, IMF etc. In this case the approach is referred to as QTL (quantitative trait locus) mapping, wherein a QTL comprises at least a part of the nucleic acid genome of an animal where genetic information capable of influencing said quantitative trait (in said animal or in its offspring) is located. Information at DNA level can not only help to fix a specific major gene in a population, but also assist in the selection of a quantitative trait which is already selected for. Molecular information in addition to phenotypic data can increase the accuracy of selection and therefore the selection response.

Improving meat quality or carcass quality is not just about changing levels of traits like tenderness or marbling, but it is also about increasing uniformity. The existence of major genes provides excellent opportunities for improving meat quality because it allows large steps to be made in the desired direction. Secondly, it will help to reduce variation, since we can fix relevant genes in our products. Another aspect is that selecting for major genes allows differentiation for specific markets. Studies are underway in several species, particularly, pigs, sheep, deer and beef cattle.

In particular, intense selection for meat production has resulted in animals with extreme muscularity and leanness in several livestock species. In recent years it has become feasible to map and clone several of the genes causing these phenotypes, paving the way towards more efficient marker assisted selection, targeted drug development (performance enhancing products) and transgenesis. Mutations in the ryanodine receptor (Fuji

et al, 1991; MacLennan and Phillips, 1993) and myostatin (Grobet et al, 1997; Kambadur et al, 1997; McPherron and Lee, 1997) have been shown to cause muscular hypertrophies in pigs and cattle respectively, while  
5 genes with major effects on muscularity and/or fat deposition have for instance been mapped to pig chromosome 4 (Andersson et al, 1994) and sheep chromosome 18 (Cocket et al, 1996).

However, although there have been successes in  
10 identifying QTLs, the information is currently of limited use within commercial breeding programmes. Many workers in this field conclude that it is necessary to identify the particular genes underlying the QTL. This is a substantial task, as the QTL region is usually relatively  
15 large and may contain many genes. Identification of the relevant genes from the many that may be involved thus remains a significant hurdle in farm animals.

The invention provides a method for selecting a  
20 domestic animal for having desired genotypic or potential phenotypic properties comprising testing said animal for the presence of a parentally imprinted qualitative or quantitative trait locus (QTL). Herein, a domestic animal is defined as an animal being selected or having been  
25 derived from an animal having been selected for having desired genotypic or potential phenotypic properties.

Domestic animals provide a rich resource of genetic and phenotypic variation, traditionally domestication involves selecting an animal or its offspring for having  
30 desired genotypic or potential phenotypic properties. This selection process has in the past century been facilitated by growing understanding and utilisation of the laws of Mendelian inheritance. One of the major problems in breeding programs of domestic animals is the  
35 negative genetic correlation between reproductive capacity and production traits. This is for example the case in cattle (a high milk production generally results



in slim cows and bulls) poultry, broiler lines have a low level of egg production and layers have generally very low muscle growth), pigs (very prolific sows are in general fat and have comparatively less meat) or sheep (high prolific breeds have low carcass quality and vice versa). The invention now provides that knowledge of the parental imprinting character of various traits allows to select for example sire lines homozygous for a paternally imprinted QTL for example linked with muscle production or growth; the selection for such traits can thus be less stringent in dam lines in favour of the reproductive quality. The phenomenon of genetic or parental imprinting has never been utilised in selecting domestic animals, it was never considered feasible to employ this elusive genetic characteristic in practical breeding programmes. The invention provides a breeding programme, wherein knowledge of the parental imprinting character of a desired trait, as demonstrated herein, results in a breeding programme, for example in a BLUP programme, with a modified animal model. This increases the accuracy of the breeding value estimation and speeds up selection compared to conventional breeding programmes. Until now, the effect of a parentally imprinted trait in the estimation of a conventional BLUP programme was neglected; using and understanding the parental character of the desired trait, as provided by the invention, allows selecting on parental imprinting, even without DNA testing. For example, selecting genes characterised by paternal imprinting is provided to help increase uniformity; a (terminal) parent homozygous for the "good or wanted" alleles will pass them to all offspring, regardless of the other parent's alleles, and the offspring will all express the desired parent's alleles. This results in more uniform offspring. Alleles that are interesting or favourable from the maternal side or often the ones that have opposite effects to alleles from the paternal side. For example, in meat animals such as pigs alleles linked with meat quality traits such as intra-

muscular fat or muscle mass could be fixed in the dam lines while alleles linked with reduced back fat could be fixed in the sire lines. Other desirable combinations are for example fertility and/or milk yield in the female  
5 line with growth rates and/or muscle mass in the male lines.

In a preferred embodiment, the invention provides a method for selecting a domestic animal for having desired genotypic or potential phenotypic properties comprising  
10 testing a nucleic acid sample from said animal for the presence of a parentally imprinted quantitative trait locus (QTL). A nucleic acid sample can in general be obtained from various parts of the animal's body by methods known in the art. Traditional samples for the  
15 purpose of nucleic acid testing are blood samples or skin or mucosal surface samples, but samples from other tissues can be used as well, in particular sperm samples, oocyte or embryo samples can be used. In such a sample, the presence and/or sequence of a specific nucleic acid,  
20 be it DNA or RNA, can be determined with methods known in the art, such as hybridisation or nucleic acid amplification or sequencing techniques known in the art. The invention provides testing such a sample for the presence of nucleic acid wherein a QTL or allele  
25 associated therewith is associated with the phenomenon of parental imprinting, for example where it is determined whether a paternal or maternal allele of said QTL is capable of being predominantly expressed in said animal.

The purpose of breeding programs in livestock is to  
30 enhance the performances of animals by improving their genetic composition. In essence this improvement accrues by increasing the frequency of the most favourable alleles for the genes influencing the performance characteristics of interest. These genes are referred to  
35 as QTL. Until the beginning of the nineties, genetic improvement was achieved via the use of biometrical methods, but without molecular knowledge of the underlying QTL.

Since the beginning of the nineties and due to recent developments in genomics, it is conceivable to identify the QTL underlying a trait of interest. The invention now provides identifying and using parentally  
5 imprinted QTLs which are useful for selecting animals by mapping quantitative trait loci. Again, the phenomenon of genetic or paternal imprinting has never been utilised in selecting domestic animals, it was never considered feasible to employ this elusive genetic characteristic in  
10 practical breeding programmes. For example Kovacs and Kloting (Biochem. Mol. Biol. Int. 44:399-405, 1998), where parental imprinting is not mentioned, and not suggested, found linkage of a trait in female rats, but not in males, suggesting a possible sex specificity  
15 associated with a chromosomal region, which of course excludes parental imprinting, a phenomenon wherein the imprinted trait of one parent is preferably but gender-aspecifically expressed in his or her offspring.

The invention provides the initial localisation of a  
20 parentally imprinted QTL on the genome by linkage analysis with genetic markers, and the actual identification of the parentally imprinted gene(s) and causal mutations therein. Molecular knowledge of such a parentally imprinted QTL allows for more efficient  
25 breeding designs herewith provided. Applications of molecular knowledge of parentally imprinted QTLs in breeding programs include: marker assisted segregation analysis to identify the segregation of functionally distinct parentally imprinted QTL alleles in the  
30 populations of interest, marker assisted selection (MAS) performed within lines to enhance genetic response by increasing selection accuracy, selection intensity or by reducing the generation interval using the understanding of the phenomenon of parental imprinting, marker assisted  
35 introgression (MAI) to efficiently transfer favourable parentally imprinted QTL alleles from a donor to a recipient population, genetic engineering of the identified parentally QTL and genetic modification of the breeding stock using transgenic technology, development

of performance enhancing products using targeted drug development exploiting molecular knowledge of said QTL.

The inventors undertook two independent experiments to determine the practical use of parental imprinting of a QTL.

In a first experiment, performed in a previously described Piétrain x Large White intercross, the likelihood of the data were computed under a model of paternal (paternal allele only expressed) and maternal imprinting (maternal allele only expressed) and compared with the likelihood of the data under a model of a conventional "Mendelian" QTL. The results strikingly demonstrated that the QTL was indeed paternally expressed, the QTL allele (Piétrain or Large White) inherited from the F<sub>1</sub> sow having no effect whatsoever on the carcass quality and quantity of the F<sub>2</sub> offspring. It was seen that very significant lodscores were obtained when testing for the presence of a paternally expressed QTL, while there was no evidence at all for the segregation of a QTL when studying the chromosomes transmitted by the sows. The same tendency was observed for all traits showing that the same imprinted gene is responsible for the effects observed on the different traits. Table 1 reports the maximum likelihood (ML) phenotypic means for the F<sub>2</sub> offspring sorted by inherited paternal QTL allele.

In a second experiment performed in the Wild Boar X Large White intercross, QTL analyses of body composition, fatness, meat quality, and growth traits was carried out with the chromosome 2 map using a statistical model testing for the presence of an imprinting effect. Clear evidence for a paternally expressed QTL located at the very distal tip of 2p was obtained (Fig. 2; Table1). The clear paternal expression of a QTL is illustrated by the least squares means which fall into two classes following the population origin of the paternally inherited allele (Table 1). For a given paternally imprinted QTL, implementation of marker assisted segregation analysis, selection (MAS) and introgression (MAI), can be performed

using genetic markers that are linked to the QTL, genetic markers that are in linkage disequilibrium with the QTL, or using the actual causal mutations within the QTL.

Understanding the parent-of-origin effect

- 5 characterising a QTL allows for its optimal use in breeding programs. Indeed, marker assisted segregation analysis under a model of parental imprinting will yield better estimates of QTL allele effects. Moreover it allows for the application of specific breeding schemes
- 10 to optimally exploit a QTL. In one embodiment of the invention, the most favourable QTL alleles would be fixed in breeding animal lines and for example used to generate commercial, crossbred males by marker assisted selection (MAS, within lines) and marker assisted introgression
- 15 (MAI, between lines). In another embodiment, the worst QTL alleles would be fixed in the animal lines used to generate commercial crossbred females by MAS (within lines) and MAI (between lines).

- In a preferred embodiment of the invention, said
- 20 animal is a pig. Note for example that the invention provides the insight that today half of the offspring from commercially popular Piétrain<sub>x</sub> Large White crossbred boars inherit an unfavourable Large White muscle mass QTL as provided by the invention causing considerable loss,
- 25 and the invention now for example provides the possibility to select the better half of the population in that respect. However, it is also possible to select commercial sow lines enriched with the in the boars unfavourable alleles, allowing to equip the sows with
- 30 other alleles more desirable for for example reproductive purposes.

- In a preferred embodiment of a method provided by the invention, said QTL is located at a position corresponding to a QTL located at chromosome 2 in the
- 35 pig. For example, it is known from comparative mapping data between pig and human, including bidirectional chromosome painting, that SSC2p is homologous to HSA11pter-q13<sup>11,12</sup>. HSA11pter-q13 is known to harbour a

cluster of imprinted genes: IGF2, INS2, H19, MAH2, P57<sup>KIP2</sup>, K<sub>v</sub>LQTL1, Tapa1,/CD81, Orctl2, Impt1 and Ip1. The cluster of imprinted genes located in HSA11pter-q13 is characterised by 8 maternally expressed genes H19, MASH2, P57<sup>KIP2</sup>, K<sub>v</sub>LQTL1, TAPA1/CD81, ORCTL2, IMPT1 and IP1, and two paternally expressed genes: IGF2 and INS. However, Johanson et al (Genomics 25:682-690, 1995) and Reik et al (Trends in Genetics, 13:330-334, 1997) show that the whereabouts of these loci in various animals are not clear. For example, the HSA11 and MMU7 loci do not correspond among each other, the MMU7 and the SSC2 loci do not correspond, whereas the HSA11 and SSC2 loci seem to correspond, and no guidance is given where one or more of for example the above identified parentally expressed individual genes are localised on the three species' chromosomes.

Other domestic animals, such as cattle, sheep, poultry and fish, having similar regions in their genome harbouring such a cluster of imprinted genes or QTLs, the invention herewith provides use of these orthologous regions of other domestic animals in applying the phenomenon of parental imprinting in breeding programmes. In pigs, said cluster is mapped at around position 2p1.7 of chromosome 2, however, a method as provided by the invention employing (fragments of) said maternally or paternally expressed orthologous or homologous genes or QTLs are advantageously used in other animals as well for breeding and selecting purposes. For example, a method is provided wherein said QTL is related to the potential muscle mass and/or fat deposition, preferably with limited effects on other traits such as meat quality and daily gain of said animal or wherein said QTL comprises at least a part of an insulin-like growth factor-2 (IGF2) allele. Reik et al (Trends in Genetics, 13:330-334, 1997) explain that this gene in humans is related to Beckwith-Wiedemann syndrome, an apparently parentally imprinted disease syndrome most commonly seen with human fetuses, where the gene has an important role in prenatal

development. No relationship is shown or suggested with postnatal development relating to muscle development or fatness in (domestic) animals.

In a preferred embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7. In particular, the invention relates to the use of genetic markers for the telomeric end of pig chromosome 2p in marker selection (MAS) of a parentally imprinted Quantitative Trait Locus (QTL) affecting carcass yield and quality in pigs. Furthermore, the invention relates to the use of genetic markers associated with the IGF2 locus in MAS in pigs, such as polymorphisms and microsatellites and other characterising nucleic acid sequences shown herein, such as shown in figures 4 to 10. In a preferred embodiment, the invention provides a QTL located at the distal tip of *Sus scrofa* chromosomes 2 with effects on varies measurements of carcass quality and quantity, particularly muscle mass and fat deposition.

In a first experiment, a QTL mapping analysis was performed in a Wild Boar X Large White intercross counting 200 F<sub>2</sub> individuals. The F<sub>2</sub> animals were sacrificed at a live eight of at least 80 kg or at a maximum age of 190 days. Phenotypic data on birth weight, growth, fat deposition, body composition, weight of internal organs, and meat quality were collected; a detailed description of the phenotypic traits are provided by Andersson et al<sup>1</sup> and Andersson-Eklund et al<sup>4</sup>.

A QTL (without any significant effect on back-fat thickness) at an unspecified locus on the proximal end of chromosome 2 with moderate effect on muscle mass, and located about 30cM away from the parentally imprinted QTL reported here, was previously reported by the inventors; whereas the QTL as now provided has a very large effect, explaining at least 20-30% of variance, making the QTL of

the present invention commercially very attractive, which is even more so because the present QTL is parentally imprinted. The marker map of chromosome 2p was improved as part of this invention by adding microsatellite markers in order to cover the entire chromosome arm. The following microsatellite markers were used: *Swc9*, *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. QTL analyses of body composition, fatness, meat quality, and growth traits were carried out with the new chromosome 2 map. Clear evidence for a QTL located at the very distal tip of 2p was obtained (Fig. 1; Table 1). The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F<sub>2</sub> population. Large effects on the area of the longissimus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population.

In a second experiment, QTL mapping was performed in a Piétrain X Large White intercross comprising 1125 F<sub>2</sub> offspring. The Large White and Piétrain parental breeds differ for a number of economically important phenotypes. Piétrains are famous for their exceptional muscularity and leanness <sup>10</sup>(Figure 2, while Large Whites show superior growth performance. Twenty-one distinct phenotypes measuring growth performance (5), muscularity (6), fat deposition (6), and meat quality (4), were recorded on all F<sub>2</sub> offspring. In order to map QTL underlying the genetic differences between these breeds, the inventors undertook a whole genome scan using microsatellite markers on an initial sample of 677 F<sub>2</sub> individuals. The following microsatellite marker map was used to analyse



chromosome 2;:SW2443, SWC9 and SW2623, SWR2516-(0,20)-  
SWR783-(0,29)-SW240-(0,20)-SW776-(0,08)-S0010-(0,04)-  
SW1695-(0,36)-SWR308. Analysis of pig chromosome 2 using  
a Maximum Likelihood multipoint algorithm, revealed  
5 highly significant lodscores (up to 20) for three of the  
six phenotypes measuring muscularity (% lean cuts, % ham,  
% loin) and three of the six phenotypes measuring fat  
deposition (back-fat thickness (BFT), % backfat, % fat  
cuts) at the distal end of the short arm of chromosome 2  
10 (Figure 1). Positive lodscores were obtained in the  
corresponding chromosome region for the remaining six  
muscularity and fatness phenotypes, however, not reaching  
the experiment-wise significance threshold ( $\alpha=5\%$ ). There  
was no evidence for an effect of the corresponding QTL on  
15 growth performance (including birth weight) or recorded  
meat quality measurements (data not shown). To confirm  
this finding, the remaining sample of 355 F<sub>2</sub> offspring was  
genotyped for the four most distal 2p markers and QTL  
analysis performed for the traits yielding the highest  
20 lodscores in the first analysis. Lodscores ranged from  
2.1 to 7.7, clearly confirming the presence of a major  
QTL in this region. Table 2 reports the corresponding ML  
estimates for the three genotypic means as well as the  
residual variance. Evidence based on marker assisted  
25 segregation analysis points towards residual segregation  
at this locus within the Piétrain population.

These experiments therefore clearly indicated  
the existence of a QTL with major effect on carcass  
quality and quantity on the telomeric end of pig  
30 chromosome arm 2p; the likely existence of an allelic  
series at this QTL with at least three alleles: Wild-Boar  
< Large White < Piétrain, and possibly more given the  
observed segregation within the Piétrain breed.

The effects of the identified QTL on muscle mass and  
35 fat deposition are truly major, being of the same  
magnitude of those reported for the CRC locus though  
apparently without the associated deleterious effects on  
meat quality. We estimate that both loci jointly explain

close to 50% of the Piétrain versus Large White breed difference for muscularity and leanness. The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F<sub>2</sub> population. Large effects on the area of the longissimus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL, when compared to the Wild Boar allele, was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population. The strong imprinting effect observed for all affected traits shows that a single causative locus is involved. The pleiotropic effects on skeletal muscle mass and the size of the heart appear adaptive from a physiological point of view as a larger muscle mass requires a larger cardiac output.

In a further embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7., wherein said QTL comprises at least a part of a *Sus scrofa* insulin-like growth factor-2 (IGF2) allele or a genonic area closely related thereto, such as polymorphisms and microsatellites and other characterising nucleic acid sequences shown herein, such as shown in figures 4 to 10. The important role of *IGF2* for prenatal development is well-documented from knock-out mice as well as from its causative role in the human Beckwith-Wiedemann syndrome. This invention demonstrates an important role for the *IGF2*-region also for postnatal development.

To show the role of *Igf2* the inventors performed the following three experiments:

A genomic *IGF2* clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone  
5 gave a strong consistent signal on the terminal part of chromosome 2p.

A polymorphic microsatellite is located in the 3'UTR of *IGF2* in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible  
10 presence of a corresponding porcine microsatellite was investigated by direct sequencing of the *IFG2* 3'UTR using the BAC clone. A complex microsatellite was identified about 800bp downstream of the stop codon; a sequence comparison revealed that this microsatellite was  
15 identical to a previously described anonymous microsatellite, *Swc9*<sup>6</sup>. This marker was used in the initial QTL mapping experiments and its location on the genetic map correspond with the most likely position of the QTL both in the Piétrain X Large White and in the Large White  
20 x Wild Boar pedigree.

Analysis of skeletal muscle and liver cDNA from 10-week old fetuses heterozygous for a nt241 (G-A) transversion in the second exon of the porcine *IGFII* gene and *SWC9*, shows that the *IGFII* gene is imprinted in these  
25 tissues in the pig as well and only expressed from the paternal allele.

Based on a published porcine adult liver cDNA sequence<sup>16</sup>, the inventors designed primer pairs allowing to amplify the entire *IgfII* coding sequence with 222 bp  
30 of leader and 280 bp of trailer sequence from adult skeletal muscle cDNA. Piétrain and Large White RT-PCR products were sequenced indication that the coding sequences are identical in both breeds and with the published sequence. However, a G~~A~~A transition was found  
35 in the leader sequence corresponding to exon 2 in man. Following conventional nomenclature, this polymorphism will be referred to as nt241(G-A). We developed a screening test for this single nucleotide polymorphism

9(SNP) based on the ligation amplification reaction (LAR), allowing us to genotype our pedigree material. Based on these data, *IgfII* was shown to colocalize with the SWC9 microsatellite marker ( $\theta=0\%$ ), therefore  
5 virtually coinciding with the most likely position of the QTL, and well within the 95% support interval for the QTL. Subsequent sequence analysis demonstrated that the microsatellite marker SWC9 is actually located within the 3'UTR of the *IgfII* gene.

10 As previously mentioned, the knowledge of this QTL provides a method for the selection of animals such as pigs with improved carcass merit. Different embodiments of the invention are envisaged, including:  
15 segregation of functionally distinct QTL alleles in the populations of interest; marker assisted selection (MAS) performed within lines to enhance genetic response by increasing selection accuracy, selection intensity or by reducing the generation interval; marker assisted  
20 introgression (MAI) to efficiently transfer favourable QTL alleles from a donor to a recipient population, thereby enhancing genetic response in the recipient population. Implementation of embodiments marker assisted segregation analysis, selection (MAS) and introgression  
25 (MAI), can be performed using genetic markers that are linked to the QTL; genetic markers that are in linkage disequilibrium with the QTL, the actual causal mutations within the QTL.

In a further embodiment, the invention provides a  
30 method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7., wherein said QTL is  
35 paternally expressed, i.e. is expressed from the paternal allele. In man and mouse, *Igf2* is known to be imprinted and to be expressed exclusively from the paternal allele in several tissues. Analysis of skeletal muscle cDNA from

pigs heterozygous for the SNP and/or SWC9, shows that the same imprinting holds in the pig as well. Understanding the parent-of-origin effect characterising the QTL as provided by the invention now allows for its optimal use in breeding programs. Indeed, today half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit the unfavourable Large White allele causing considerable loss. Using a method as provide by the invention avoids this problem.

10 The invention furthermore provides an isolated and/or recombinant nucleic acid or functional fragment derived thereof comprising a parentally imprinted quantitative trait locus (QTL) or fragment thereof capable of being predominantly expressed by one parental  
15 allele. Having such a nucleic acid as provided by the invention available allows constructing transgenic animals wherein favourable genes are capable of being exclusively or predominantly expressed by one parental allele, thereby equipping the offspring of said animal  
20 homozygous for a desired trait with desired properties related to that parental allele that is expressed.

In a preferred embodiment, the invention provides an isolated and/or recombinant nucleic acid or fragment derived thereof comprising a synthetic parentally  
25 imprinted quantitative trait locus (QTL) or functional fragment thereof derived from at least one chromosome. Synthetic herein describes a parentally expressed QTL wherein various elements are combined that originate from distinct locations from the genome of one or more  
30 animals. The invention provides recombinant nucleic acid wherein sequences related to parental imprinting of one QTL are combined with sequences relating to genes or favourable alleles of a second QTL. Such a gene construct is favourably used to obtain transgenic animals wherein  
35 the second QTL has been equipped with paternal imprinting, as opposed to the inheritance pattern in the native animal from which the second QTL is derived. Such a second QTL can for example be derived from the same

chromosome where the parental imprinting region is located, but can also be derived from a different chromosome from the same or even a different species. In the pig, such a second QTL can for example be related to an oestrogen receptor (ESR)-gene (Rothschild et al, PNAS, 93, 201-201, 1996) or a FAT-QTL (Andersson, Science, 263, 1771-1774, 1994) for example derived from an other pig chromosome, such as chromosome 4. A second or further QTL can also be derived from another (domestic) animal or a human.

The invention furthermore provides an isolated and/or recombinant nucleic acid or functional fragment derived thereof at least partly corresponding to a QTL of a pig located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7 wherein said QTL is related to the potential muscle mass and/or fat deposition of said pig and/or wherein said QTL comprises at least a part of a *Sus scrofa* insulin-like growth factor-2 (IGF2) allele, preferably at least spanning a region between INS and H19, or preferably derived from a domestic pig, such as a Pietrain, Meishan, Duroc, Landrace or Large White, or from a Wild Boar. For example, a genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong consistent signal on the terminal part of chromosome 2p. A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IGF2 3'UTR using the BAC clone. A complex microsatellite was identified about 800 bp downstream of the stop codon; a sequence comparison revealed that this microsatellite is identical to a previously described anonymous microsatellite, Swc9. PCR primers were designed and the microsatellite (IGF2ms) was found to be highly polymorphic with three different alleles among the two Wild Boar founders and another two

among the eight Large White founders. *IGF2ms* was fully informative in the intercross as the breed of origin as well as the parent of origin could be determined with confidence for each allele in each F<sub>2</sub> animal.

5        A linkage analysis using the intercross pedigree was carried out with *IGF2ms* and the microsatellites *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. *IGF2* was firmly assigned to 2p by highly significant lod scores (e.g. Z=89.0,  $\theta=0.003$  against *Swr2516*). Multipoint  
10 analyses, including previously typed chromosome 2 markers, revealed the following order of loci (sex-average map distances in Kosambi cM): *Sw2443/Swr2516*-0.3-*IGF2*-14.9-*Sw2623*-10.3-*Sw256*. No recombinant was observed  
15 location of *IGF2* in relation to these loci is based on a single recombinant giving a lod score support of 0.8 for the reported order. The most distal marker in our previous QTL study, *Sw256*, is located about 25 cM from the distal end of the linkage group.

20        The invention furthermore provides use of a nucleic acid or functional fragment derived thereof according to the invention in a method according to the invention. In a preferred embodiment, use of a method according to invention is provided to select a breeding animal or  
25 animal destined for slaughter, or embryos or semen derived from these animals for having desired genotypic or potential phenotypic properties. In particular, the invention provides such use wherein said properties are related to muscle mass and/or fat deposition. The QTL as  
30 provided by the invention may be exploited or used to improve for example lean meat content or back-fat thickness by marker assisted selection within populations or by marker assisted introgression of favorable alleles from one population to another. Examples of marker  
35 assisted selection using the QTL as provided by the invention are use of marker assisted segregation analysis

with linked markers or with markers in disequilibrium to identify functionally distinct QTL alleles. Furthermore, identification of a causative mutation in the QTL is now possible, again leading to identify functionally distinct QTL alleles. Such functionally distinct QTL alleles located at the distal tip of chromosome 2p with large effects on skeletal muscle mass, the size of the heart, and on back-fat thickness are also provided by the invention. The observation of a similar QTL effect in a Large White x Wild Boar as well as in a Piétrain x Large White intercross provides proof of the existence of a series of at least three distinct functional alleles. Moreover, preliminary evidence based on marker assisted segregation analysis points towards residual segregation at this locus within the Piétrain population (data not shown). The occurrence of an allelic series as provided by the invention allows identifying causal polymorphisms which - based on the quantitative nature of the observed effect - are unlikely to be gross gene alterations but rather subtle regulatory mutations. The effects on muscle mass of the three alleles rank in the same order as the breeds in which they are found i.e. Piétrain pigs are more muscular than Large White pigs that in turn have higher lean meat content than Wild Boars. The invention furthermore provides use of the alleles as provided by the invention for within line selection or for marker assisted introgression using linked markers, markers in disequilibrium or alleles comprising causative mutations.

The invention furthermore provides an animal selected by using a method according to the invention. For example, a pig characterised in being homozygous for an allele in a QTL located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7 can now be selected and is thus provided by the invention. Since said QTL is related to the potential muscle mass and/or fat deposition of said pig and/or said QTL comprises at least a part of a *Sus scrofa* insulin-like growth factor-2 (IGF2) allele, it is



possible to select promising pigs to be used for breeding or to be slaughtered. In particular an animal according to the invention which is a male is provided. Such a male, or its sperm or an embryo derived thereof can advantageously be used in breeding animals for creating breeding lines or for finally breeding animals destined for slaughter. In a preferred embodiment of such use as provided by the invention, a male, or its sperm, deliberately selected for being homozygous for an allele causing the extreme muscular hypertrophy and leanness, is used to produce offspring heterozygous for such an allele. Due to said allele's paternal expression, said offspring will also show the favourable traits for example related to muscle mass, even if the parent female has a different genetic background. Moreover, it is now possible to positively select the female(s) for having different traits, for example related to fertility, without having a negative effect on the muscle mass trait that is inherited from the allele from the selected male. For example, earlier such males could occasionally be seen with Piétrain pigs but genetically it was not understood how to most profitably use these traits in breeding programmes.

Furthermore, the invention provides a transgenic animal, sperm and an embryo derived thereof, comprising a synthetic parentally imprinted QTL or functional fragment thereof as provided by the invention, i.e. it is provided by the invention to introduce a favourable recombinant allele; for example introduce the oestrogen receptor locus related to increased litter size of an animal homozygously in a parentally imprinted region of a grandparent animal (for example the father of a hybrid sow if the region was paternally imprinted and the grandparent was a boar); to introduce a favourable fat-related allele or muscle mass-related recombinant allele in a paternally imprinted region, and so on. Recombinant alleles that are interesting or favourable from the maternal side or often the ones that have opposite effects to alleles from the paternal side. For example,

in meat animals such as pigs recombinant alleles linked with meat quality traits such as intra-muscular fat or muscle mass could be fixed in the dam lines while recombinant alleles linked with reduced back fat could be fixed in the sire lines. Other desirable combinations are for example fertility and/or milk yield in the female line with growth rates and/or muscle mass in the male lines.

The invention is further explained in the detailed description without limiting the invention.

Detailed description.

#### Example 1: Wild Boar x Large White intercrosses

##### Methods

Isolation of an *IGF2* BAC clone and fluorescent *in situ* hybridization (FISH). *IGF2* primers (F:5'-GGCAAGTTCTTCCGCTAATGA-3' and R:5'-GCACCGCAGAATTACGACAA-3') for PCR amplification of a part of the last exon and 3'UTR were designed on the basis of a porcine *IGF2* cDNA sequence (GenBank X56094). The primers were used to screen a porcine BAC library and the clone 253G10 was isolated. Crude BAC DNA was prepared as described<sup>24</sup>. The BAC DNA was linearized with *EcoRV* and purified with QIAEXII (QIAGEN GmbH, Germany). The clone was labeled with biotin-14-dATP using the GIBCO-BRL Bionick labeling system (BRL18246-015). Porcine metaphase chromosomes were obtained from pokeweed (Seromed) stimulated lymphocytes using standard techniques. The slides were aged for two days at room temperature and then kept at -20°C until use. FISH analysis was carried out as previously described<sup>25</sup>. The final concentration of the probe in the hybridization mix was 10 ng/μl. Repetitive sequences were suppressed with standard concentrations of porcine

genomic DNA. After post-hybridization washing, the biotinylated probe was detected with two layers of avidin-FITC (Vector A-2011). The chromosomes were counterstained with 0.3 mg/ml DAPI (4,6-Diamino-2-phenylindole; Sigma D9542), which produced a G-banding like pattern. No posthybridization banding was needed, since chromosome 2 is easily recognized without banding. A total of 20 metaphase spreads were examined under an Olympus BX-60 fluorescence microscope connected to an IMAC-CCD S30 video camera and equipped with an ISIS 1.65 (Metasystems) software.

Sequence, microsatellite, and linkage analysis.

About two µg of linearized and purified BAC DNA was used for direct sequencing with 20 pmoles of primers and BigDye Terminator chemistry (Perkin Elmer, USA). DNA sequencing was done from the 3' end of the last exon towards the 3' end of the UTR until a microsatellite was detected. A primer set (F:5'-GTTTCTCCTGTACCCACACGCATCCC-3' and R:5'-Fluorescein-CTACAAGCTGGGCTCAGGG-3') was designed for the amplification of the *IGF2* microsatellite which is about 250 bp long and located approximately 800 bp downstream from the stop codon. The microsatellite was PCR amplified using fluorescently labeled primers and the genotyping was carried out using an ABI377 sequencer and the GeneScan/Genotyper softwares (Perkin Elmer, USA). Two-point and multipoint linkage analysis were done with the Cri-Map software<sup>26</sup>.

30

Animals and phenotypic data.

The intercross pedigree comprised two European Wild Boar males and eight Large White females, 4 F<sub>1</sub> males and 22 F<sub>1</sub> females, and 200 F<sub>2</sub> progeny<sup>1</sup>. The F<sub>2</sub> animals were sacrificed at a live weight of at least 80 kg or at a

maximum age of 190 days. Phenotypic data on birth weight, growth, fat deposition, body composition, weight of internal organs, and meat quality were collected; a detailed description of the phenotypic traits are  
5 provided by Andersson *et al.*<sup>1</sup> and Andersson-Eklund *et al.*<sup>4</sup>

Statistical analysis.

10 Interval mapping for the presence of QTL were carried out with a least squares method developed for the analysis of crosses between outbred lines<sup>27</sup>. The method is based on the assumption that the two divergent lines are fixed for alternative QTL alleles. There are four possible  
15 genotypes in the F<sub>2</sub> generation as regards the grandparental origin of the alleles at each locus. This makes it possible to fit three effects: additive, dominance, and imprinting<sup>2</sup>. The latter is estimated as the difference between the two types of heterozygotes,  
20 the one receiving the Wild Boar allele through an F<sub>1</sub> sire and the one receiving it from an F<sub>1</sub> dam. An F-ratio was calculated using this model (with 3 d.f.) versus a reduced model without a QTL effect for each cM of chromosome 2. The most likely position of a QTL was  
25 obtained as the location giving the highest F-ratio. Genome-wise significance thresholds were obtained empirically by a permutation test<sup>28</sup> as described<sup>2</sup>. The QTL model including an imprinting effect was compared with a model without imprinting (with 1 d.f.) to test  
30 whether the imprinting effect was significant.

The statistical models also included the fixed effects and covariates that were relevant for the respective traits; see Andersson-Eklund *et al.*<sup>4</sup> for a more detailed description of the statistical models used.  
35 Family was included to account for background genetic

effects and maternal effects. Carcass weight was included as a covariate to discern QTL effects on correlated traits, which means that all results concerning body composition were compared at equal weights. Least-squares means for each genotype class at the *IGF2* locus were estimated with a single point analysis using Procedure GLM of SAS<sup>29</sup>; the model included the same fixed effects and covariates as used in the interval mapping analyses. The QTL shows a clear parent of origin-specific expression and the map position coincides with that of the insulin-like growth factor II gene (*IGF2*), indicating *IGF2* as the causative gene. A highly significant segregation distortion (excess of Wild Boar-derived alleles) was also observed at this locus. The results demonstrate an important effect of the *IGF2* region on postnatal development and it is possible that the presence of a paternally expressed *IGF2*-linked QTL in humans and in rodent model organisms has so far been overlooked due to experimental design or statistical treatment of data. The study has also important implications for quantitative genetics theory and practical pig breeding.

*IGF2* was identified as a positional candidate gene for this QTL due to the observed similarity between pig chromosome 2p and human chromosome 11p. A genomic *IGF2* clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong consistent signal on the terminal part of chromosome 2p (Fig. 1). A polymorphic microsatellite is located in the 3'UTR of *IGF2* in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the *IGF2* 3'UTR using the BAC clone. A complex microsatellite was identified about 800 bp downstream of the stop codon; a sequence comparison revealed that this microsatellite is identical

to a previously described anonymous microsatellite, *Swc96*. PCR primers were designed and the microsatellite (*IGF2ms*) was found to be highly polymorphic with three different alleles among the two Wild Boar founders and another two among the eight Large White founders. *IGF2ms* was fully informative in the intercross as the breed of origin as well as the parent of origin could be determined with confidence for each allele in each  $F_2$  animal.

10 A linkage analysis using the intercross pedigree was carried out with *IGF2ms* and the microsatellites *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. *IGF2* was firmly assigned to 2p by highly significant lod scores (e.g.  $Z=89.0$ ,  $\theta=0.003$  against *Swr2516*). Multipoint  
15 analyses, including previously typed chromosome 2 markers<sup>8</sup>, revealed the following order of loci (sex-average map distances in Kosambi cM): *Sw2443/Swr2516*-0.3-*IGF2*-14.9-*Sw2623*-10.3-*Sw256*. No recombinant was observed between *Sw2443* and *Swr2516*, and the suggested proximal  
20 location of *IGF2* in relation to these loci is based on a single recombinant giving a lod score support of 0.8 for the reported order. The most distal marker in our previous QTL study, *Sw256*, is located about 25 cM from the distal end of the linkage group.

25 QTL analyses of body composition, fatness, meat quality, and growth traits were carried out with the new chromosome 2 map using a statistical model testing for the possible presence of an imprinting effect as expected for *IGF2*. Clear evidence for a paternally expressed QTL  
30 located at the very distal tip of 2p was obtained (Fig. 2; Table 1). The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the  $F_2$  population. Large effects on the area of the longissimus dorsi muscle, on  
35 the weight of the heart, and on back-fat thickness

(subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population. The strong imprinting effect observed for all affected traits strongly suggests a single causative locus. The pleiotropic effects on skeletal muscle mass and the size of the heart appear adaptive from a physiological point of view as a larger muscle mass requires a larger cardiac output. The clear paternal expression of this QTL is illustrated by the least squares means which fall into two classes following the population origin of the paternally inherited allele (Table 1). It is worth noticing though that there was a non-significant trend towards less extreme values for the two heterozygous classes, in particular for the estimated effect on the area of longissimus dorsi. This may be due to chance, but could have a biological explanation, e.g. that there is some expression of the maternally inherited allele or that there is a linked, non-imprinted QTL with minor effects on the traits in question.

The *IGF2*-linked QTL and the *FAT1* QTL on chromosome 4<sup>1, 9</sup> are by far the two loci with the largest effect on body composition and fatness segregating in this Wild Boar intercross. The *IGF2* QTL controls primarily muscle mass whereas *FAT1* has major effects on fat deposition including abdominal fat, a trait that was not affected by the *IGF2* QTL (Fig. 2). No significant interaction between the two loci was indicated and they control a very large proportion of the residual phenotypic variance in the F<sub>2</sub> generation. A model including both QTLs explains 33.1% of the variance for percentage lean meat in ham, 31.3% for the percentage of lean meat plus bone in back, and 26.2%

for average back fat depth (compare with a model including only chromosome 2 effects, Table 1). The two QTLs must have played a major role in the response during selection for lean growth and muscle mass in the Large White domestic pig.

A highly significant segregation distortion was observed in the *IGF2* region (excess of Wild Boar-derived alleles) as shown in Table 1 ( $\chi^2=11.7$ , d.f.=2;  $P=0.003$ ). The frequency of Wild Boar-derived *IGF2* alleles was 59% in contrast to the expected 50% and there was twice as many "Wild Boar" as "Large White" homozygotes. This deviation was observed with all three loci at the distal tip and is thus not due to typing errors. The effect was also observed with other loci but the degree of distortion decreased as a function of the distance to the distal tip of the chromosome. Blood samples for DNA preparation were collected at 12 weeks of age and we are convinced that the deviation from expected Mendelian ratios was present at birth as the number of animals lost prior to blood sampling was not sufficient to cause a deviation of this magnitude. No other of the more than 250 loci analyzed in this pedigree show such a marked segregation distortion (L. Andersson, unpublished). The segregation distortion did not show an imprinting effect, as the frequencies of the two reciprocal types of heterozygotes were identical (Table 1). This does not exclude the possibility that the QTL effects and the segregation distortion are controlled by the same locus. The segregation distortion maybe due to meiotic drive favoring the paternally expressed allele during gametogenesis, as the  $F_1$  parents were all sired by Wild Boar males. Another possibility is that the segregation distortion may be due to codominant expression of the maternal and paternal allele in some tissues and/or during a critical period of embryo development. Biallelic *IGF2* expression has been reported to occur to some extent



during human development<sup>10, 11</sup> and interestingly a strong influence of the parental species background on *IGF2* expression was recently found in a cross between *Mus musculus* and *Mus spretus*<sup>12</sup>. It is also interesting that a VNTR polymorphism at the insulin gene, which is very closely linked to *IGF2*, is associated with size at birth in humans<sup>13</sup>. It is possible that the *IGF2*-linked QTL in pigs has a minor effect on birth weight but in our data it was far from significant (Fig. 2) and there was no indication of an imprinting effect.

This study is an advance in the general knowledge concerning the biological importance of the *IGF2* locus. The important role of *IGF2* for prenatal development is well-documented from knock-out mice<sup>14</sup> as well as from its causative role in the human Beckwith-Wiedemann syndrome<sup>15</sup>. This study demonstrates an important role for the *IGF2*-region also for postnatal development. It should be stressed that our intercross between outbred populations is particularly powerful to detect QTL with a parent of origin-specific effect on a multifactorial trait. This is because multiple alleles (or haplotypes) are segregating and we could deduce whether a heterozygous F<sub>2</sub> animal received the Wild Boar allele from the F<sub>1</sub> male or female. It is quite possible that the segregation of a paternally expressed *IGF2*-linked QTL affecting a trait like obesity has been overlooked in human studies or in intercrosses between inbred rodent populations because of experimental design or statistical treatment of data. An imprinting effect cannot be detected in an intercross between two inbred lines as only two alleles are segregating at each locus. Our result has therefore significant bearings on the future analysis of the association between genetic polymorphism in the *insulin-IGF2* region and Type I diabetes<sup>16</sup>, obesity<sup>17</sup>, and variation in birth weight<sup>13</sup> in humans, as

well as for the genetic dissection of complex traits using inbred rodent models. A major impetus for generating an intercross between the domestic pig and its wild ancestor was to explore the possibilities to map and identify major loci that have responded to selection. We have now showed that two single QTLs on chromosome 2 (this study) and 4<sup>1, 2</sup> explain as much as one third of the phenotypic variance for lean meat content in the F<sub>2</sub> generation. This is a gross deviation from the underlying assumption in the classical infinitesimal model in quantitative genetics theory namely that quantitative traits are controlled by an infinite number of loci each with an infinitesimal effect. If a large proportion of the genetic difference between two divergent populations (e.g. Wild Boar and Large White) is controlled by a few loci, one would assume that selection would quickly fix QTL alleles with large effects leading to a selection plateau. However, this is not the experience in animal breeding programs or selection experiments where good persistent long-term selection responses are generally obtained, provided that the effective population size is reasonably large<sup>18</sup>. A possible explanation for this paradox is that QTL alleles controlling a large proportion of genetic differences between two populations may be due to several consecutive mutations; this may be mutations in the same gene or at several closely linked genes affecting the same trait. It has been argued that new mutations contribute substantially to long-term selection responses<sup>19</sup>, but the genomic distribution of such mutations are unknown.

The search for a single causative mutation is the paradigm as regards the analysis of genetic defects in mice and monogenic disorders in humans. We propose that this may not be the case for loci that have been under selection for a large number of generations in domestic animals, crops, or natural populations. This hypothesis

predicts the presence of multiple alleles at major QTL. It gains some support from our recent characterization of porcine coat color variation. We have found that both the alleles for dominant white color and for black-spotting  
5 differ from the corresponding wild-type alleles by at least two consecutive mutations with phenotypic effects at the *KIT* and *MC1R* loci, respectively<sup>20, 21</sup>. In this context it is highly interesting that in the accompanying example we have identified a third allele at the *IGF2*-  
10 linked QTL. The effects on muscle mass of the three alleles rank in the same order as the breeds in which they are found i.e. Piétrain pigs are more muscular than Large White pigs that in turn have higher lean meat content than Wild Boars.

15       There are good reasons to decide that *IGF2* is the causative gene for the now reported QTL. Firstly, there is a perfect agreement in map localization (Fig. 2). Secondly, it has been shown that *IGF2* is paternally expressed in mice, humans, and now in pigs, like the QTL.  
20 There are several other imprinted genes in the near vicinity of *IGF2* in mice and humans (*Mash2*, *INS2*, *H19*, *KVLQT1*, *TAPA1/CD81*, and *CDKN1C/p57<sup>KIP2</sup>*) but only *IGF2* is paternally expressed in adult tissues<sup>22</sup>. We believe that this locus provides a unique opportunity for molecular  
25 characterization of a QTL. The clear paternal expression can be used to exclude genes that do not show this mode of inheritance. Moreover, the presence of an allelic series should facilitate the difficult distinction between causative mutations and linked neutral  
30 polymorphism. We have already shown that there is no difference in coding sequence between *IGF2* alleles from Piétrain and Large White pigs suggesting that the causative mutations occur in regulatory sequences. An obvious step is to sequence the entire *IGF2* gene and its  
35 multiple promoters from the three populations. The recent

report that a VNTR polymorphism in the promoter region of the insulin (*INS*) gene affects *IGF2* expression<sup>23</sup> suggests that the causative mutations may be at a considerable distance from the *IGF2* coding sequence.

- 5           The results have several important implications for the pig breeding industry. They show that genetic imprinting is not an esoteric academic question but need to be considered in practical breeding programs. The detection of three different alleles in Wild Boar, Large
- 10   White, and Piétrain populations indicates that further alleles at the *IGF2*-linked QTL segregate within commercial populations. The paternal expression of the QTL facilitates its detection using large paternal half-sib families as the female contribution can be ignored.
- 15   The QTL is exploited to improve lean meat content by marker assisted selection within populations or by marker assisted introgression of favorable alleles from one population to another.

## Example 2: Piétrain x Large White intercrosses

## Methods

- Pedigree material:* The pedigree material utilized to map
- 5 QTL was selected from a previously described Piétrain x Large White F2 pedigree comprising > 1,800 individuals<sup>6,7</sup>. To assemble this F2 material, 27 Piétrain boars were mated to 20 Large White sows to generate an F1 generation comprising 456 individuals. 31 F1 boars were mated to
- 10 unrelated 82 F1 sows from 1984 to 1989, yielding a total of 1862 F2 offspring. F1 boars were mated on average to 7 females, and F1 sows to an average of 2,7 males. Average offspring per boar were 60 and per sow 23.
- 15 *Phenotypic information: (i) Data collection:* A total of 21 distinct phenotypes were recorded in the F2 generation<sup>6,7</sup>. These included:
- five growth traits: birth weight (g), weaning weight (Kg), grower weight (Kg), finisher weight (Kg) and
  - 20 average daily gain (ADG; Kg/day; grower to finisher period);
  - two body proportion measurements: carcass length (cm); and a conformation score (0 to 10 scale; ref.6);
  - ten measurements of carcass composition obtained by
  - 25 dissection of the chilled carcasses 24 hours after slaughter. These include measurements of muscularity: % ham (weight hams/carcass weight), % loin (weight loin/carcass weight), % shoulder (weight
  - 30 shoulder/carcass weight), % lean cuts (% ham + %loin + % shoulder); and measurements of fatness: average back-fat thickness (BFT; cm), % backfat (weight backfat/carcass weight), % belly (weight belly/carcass weight), % leaf fat (weight leaf fat/carcass weight), % jowl (weight jowl/carcass weight), and "% fat cuts" (% backfat + %
  - 35 belly + % leaft fat + % jowl).
  - four meat quality measurements: pH<sub>LD1</sub> (*Longissimus dorsi* 1

hour after slaughter), pH<sub>LD24</sub> (*Longissimus dorsi* 24 hours after slaughter), pH<sub>G1</sub> (*Gracilis* 1 hour after slaughter) and pH<sub>G24</sub> (*Gracilis* 24 hours after slaughter). (ii) *Data processing*: Individual phenotypes were preadjusted for fixed effects (sire, dam, CRC genotype, sex, year-season, parity) and covariates (litter size, birth weight, weaning weight, grower weight, finisher weight) that proved to significantly affect the corresponding trait. Variables included in the model were selected by stepwise regression.

10

*Marker genotyping*: Primer pairs utilized for PCR amplification of microsatellite markers are as described<sup>19</sup>. Marker genotyping was performed as previously described<sup>20</sup>. Genotypes at the *CRC* and *MyoD* loci were determined using conventional methods as described<sup>1,12</sup>. The LAR test for the *Igf2* SNP was developed according to Baron et al.<sup>21</sup> using a primer pair for PCR amplification (5'-CCCCTGAACTTGAGGACGAGCAGCC-3'; 5'-ATCGCTGTGGGCTGGGTGGGCTGCC-3') and a set of three primers for the LAR step (5'-FAM-CGCCCCAGCTGCCCCCAG-3'; 5'-HEX-CGCCCCAGCTGCCCCCAA-3'; 5'-CCTGAGCTGCAGCAGGCCAG-3').

20

*Map construction*: Marker maps were constructed using the TWOPOINT, BUILD and CHROMPIC options of the CRIMAP package<sup>22</sup>. To allow utilisation of this package, full-sib families related via the boar or sow were disconnected and treated independently. By doing so, some potentially usable information was neglected, yielding, however, unbiased estimates of recombination rates.

30

*QTL mapping*: (i) *Mapping Mendelian QTL*: Conventional QTL mapping was performed using a multipoint maximum likelihood method. The applied model assumed one segregating QTL per

chromosome, and fixation of alternate QTL alleles in the respective parental lines, Piétrain (P) and Large White (LW). A specific analysis program had to be developed to account for the missing genotypes of the parental generation, resulting in the fact that the parental origin of the F1 chromosomes could not be determined. Using a typical "interval mapping" strategy, an hypothetical QTL was moved along the marker map using user-defined steps. At each position, the likelihood ( $L$ ) of the pedigree data was computed as:

$$L = \sum_{\varphi=1}^{2^r} \prod_{i=1}^n \sum_{G=1}^4 (P(G|M_i, \theta, \varphi) P(y_i|G))$$

$P$  or right chromosome  $P$ ), there is a total of  $2^r$  combinations for  $r$  F1 parents.

15  $\prod_{i=1}^n$   $n$  F2

$\sum_{G=1}^4$   $i$ th F2 offspring, over the four possible QTL genotypes:

$P/P$ ,  $P/LW$ ,  $LW/P$  and  $LW/LW$

$P(G|M_i, \theta, \varphi)$   $M_i$ : the marker genotype of the  $i$ th F2 offspring and its F1 parents, (ii) : the vector of recombination rates between adjacent markers and between the hypothetical QTL and its flanking markers, and (iii)  $\theta$  the considered marker-QTL phase combination of the F1 parents.

Recombination rates and marker linkage phase of F1 parents are assumed to be known when computing this probability. Both were determined using CRIMAP in the map construction phase (see above).

$P(y_i|G)$   $y_i$ ) of offspring  $i$ , given the QTL genotype under consideration. This probability is computed from the normal density function:

$$P(y_i|G) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(y_i - \mu_G)^2}{2\sigma^2}}$$

$\mu_G$  is the phenotypic mean of the considered QTL genotype (PP, PL, LP or LL) and  $\sigma^2$  the residual variance  $\sigma^2$  was considered to be the same for the four QTL genotypic classes.

- 5 The values of  $\mu_{PP}$ ,  $\mu_{PL}=\mu_{LP}$ ,  $\mu_{LL}$  and  $\sigma^2$  maximizing  $L$  were determined using the GEMINI optimisation routine<sup>23</sup>.

The likelihood obtained under this alternative  $H_1$  hypothesis was compared with the likelihood obtained under the null hypothesis  $H_0$  of no QTL, in which the phenotypic means of the  
 10 four QTL genotypic classes were forced to be identical. The difference between the logarithms of the corresponding likelihoods yields a lodscore measuring the evidence in favour of a QTL at the corresponding map position.

- (ii) *Significance thresholds*: Following Lander & Botstein<sup>24</sup>,  
 15 lodscore thresholds ( $T$ ) associated with a chosen genome-wide significance level, were computed such that:

$$\alpha = (C + 9.21GT)\chi^2_2(4.6T)$$

$C$  corresponds to the number of chromosomes (= 19),  $G$  corresponds to the length of the genome in Morgans (= 29),

- 20 and  $\chi^2_2(4.6T)$  denotes one minus the cumulative distribution function of the chi-squared distribution with 2 d.f. Single point  $2\ln(LR)$  were assumed to be distributed as a chi-squared distribution with two degrees of freedom, as we were fitting both an additive and dominance component. To account for the  
 25 fact that we were analysing multiple traits, significance levels were adjusted by applying a Bonferoni correction corresponding to the effective number of independent traits that were analyzed. This effective number was estimated at 16 following the approach described by Spelman et al.<sup>25</sup>.  
 30 Altogether, this allowed us to set the lodscore threshold associated with an experiment-wise significance level of 5%



at 5.8. When attempting to confirm the identified QTL in an independent sample, the same approach was used, however, setting C at 1, G at 25cM and correcting for the analysis of 4.5 independent traits (as only six traits were analyzed in this sample). This yielded a lodscore threshold associated with a Type I error of 5% of 2.

(iii). *Testing for an imprinted QTL*: To test for an imprinted QTL, we assumed that only the QTL alleles transmitted by the parent of a given sex would have an effect on phenotype, the QTL alleles transmitted by the other parent being "neutral". The likelihood of the pedigree data under this hypothesis was computed using equation 1. To compute  $P(y_i | G)$ , however, the phenotypic means of the four QTL genotypes were set at  $\mu_{PP} = \mu_{PL} = \mu_P$  and  $\mu_{LP} = \mu_{LL} = \mu_L$  to test for a QTL for which the paternal allele only is expressed, and  $\mu_{PP} = \mu_{LP} = \mu_P$  and  $\mu_{PL} = \mu_{LL} = \mu_L$  to test for a QTL for which the maternal allele only is expressed. It is assumed in this notation that the first subscript refers to the paternal allele, the second subscript to the maternal allele.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  testing the presence of a Mendelian QTL;  $H_2$  testing the presence of a paternally expressed QTL, and  $H_3$  testing the presence of a maternally expressed QTL.

*RT-PCR*: Total RNA was extracted from skeletal muscle according to Chirgwin et al.<sup>26</sup>. RT-PCR was performed using the Gene-Amp RNA PCR Kit (Perkin-Elmer) The PCR products were purified using QiaQuick PCR Purification kit (Qiagen) and sequenced using Dye terminator Cycle Sequencing Ready Reaction (Perkin Elmer) and an ABI373 automatic sequencer.

In example 2 we report the identification of a QTL with major effect on muscle mass and fat deposition mapping to porcine 2p1.7. The QTL shows clear evidence for parental imprinting strongly suggesting the involvement of the *Igf2* locus.

5        A Piétrain X Large White intercross comprising 1125 F<sub>2</sub> offspring was generated as described<sup>6,7</sup>. The Large White and Piétrain parental breeds differ for a number of economically important phenotypes. Piétrains are famed for their exceptional muscularity and leanness<sup>8</sup> (Figure 2), while Large  
10        Whites show superior growth performance. Twenty-one distinct phenotypes measuring (i) growth performance (5), (ii) muscularity (6), (iii) fat deposition (6), and (iv) meat quality (4), were recorded on all F<sub>2</sub> offspring.

         In order to map QTL underlying the genetic differences  
15        between these breeds, we undertook a whole genome scan using microsatellite markers on an initial sample of 677 F<sub>2</sub> individuals. Analysis of pig chromosome 2 using a ML multipoint algorithm, revealed highly significant lodscores (up to 20) for six of the 12 phenotypes measuring muscularity  
20        and fat deposition at the distal end of the short arm of chromosome 2 (Figure 3a). Positive lodscores were obtained for the remaining six phenotypes, however, not reaching the genome-wide significance threshold ( $\alpha = 5\%$ ). To confirm this finding, the remaining sample of 355 F<sub>2</sub> offspring was  
25        genotyped for the five most distal 2p markers and QTL analysis performed for the traits yielding the highest lodscores in the first analysis. Lodscores ranged from 2.1 to 7.7, clearly confirming the presence of a major QTL in this region. Table 2 reports the corresponding ML estimates for  
30        the three genotypic means as well as the corresponding residual variance.

         Bidirectional chromosome painting establishes a correspondence between SSC2p and HSA11pter-q13<sup>9,10</sup>. At least

two serious candidate genes map to this region in man: the myogenic basic helix-loop-helix factor, *MyoD*, maps to HSA11p15.4, while *Igf2* maps to HSA11p15.5. *MyoD* is a well known key regulator of myogenesis and is one of the first myogenic markers to be switched on during development<sup>11</sup>. A previously described amplified sequence polymorphism in the porcine *MyoD* gene<sup>12</sup> proved to segregate in our F<sub>2</sub> material, which was entirely genotyped for this marker. Linkage analysis positioned the *MyoD* gene in the SW240-SW776 (odds > 1000) interval, therefore well outside the lod-2 drop off support interval for the QTL (figure 1). *Igf2* is known to enhance both proliferation and differentiation of myoblasts *in vitro*<sup>13</sup> and to cause a muscular hypertrophy when overexpressed *in vivo*. Based on a published porcine adult liver cDNA sequence<sup>14</sup>, we designed primer pairs allowing us to amplify the entire *Igf2* coding sequence with 222 bp of leader and 280 bp of trailer sequence from adult skeletal muscle cDNA. Piétrain and Large White RT-PCR products were sequenced indicating that the coding sequences was identical in both breeds and with the published sequence. However, a G A transition was found in the leader sequence corresponding to exon 2 in man (Figure 4). We developed a screening test for this single nucleotide polymorphism (SNP) based on the ligation amplification reaction (LAR), allowing us to genotype our pedigree material. Based on these data, *Igf2* was shown to colocalize with the SWC9 microsatellite marker (= 0%), therefore located at approximately 2 centimorgan from the most likely position of the QTL and well within the 95% support interval for the QTL (figure 1). Subsequent sequence analysis demonstrated that the microsatellite marker SWC9 is actually located within the 3' UTR of the *Igf2* gene. Combined with available comparative mapping data for the PGA and FSH loci, these results suggest the occurrence of an interstitial

inversion of a chromosome segment containing *MyoD*, but not *Igf2* which has remained telomeric in both species.

*Igf2* therefore appeared as a strong positional allele having the observed QTL effect. In man and mouse, *Igf2* is known to be imprinted and to be expressed exclusively from the paternal allele in several tissues<sup>15</sup>. Analysis of skeletal muscle cDNA from pigs heterozygous for the SNP and/or SWC9, shows that the same imprinting holds in this tissue in the pig as well (Figure 4). Therefore if *Igf2* were responsible for the observed effect, and knowing that only the paternal *Igf2* allele is expressed, one can predict that (i) the paternal allele transmitted by F1 boars (P or LW) would have an effect on phenotype of F2 offspring, (ii) the maternal allele transmitted by F1 sows (P or LW) would have no effect on phenotype of F2 offspring, and (iii) the likelihood of the data would be superior under a model of a bimodal (1:1) F2 population sorted by inherited paternal allele when compared to a conventional "Mendelian" model of a trimodal (1:2:1) F2 population. The QTL mapping programs were adapted in order to allow testing of the corresponding hypotheses.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  as testing for the presence of a Mendelian QTL,  $H_2$  as testing for the presence of a paternally expressed QTL, and  $H_3$  as testing for the presence of a maternally expressed QTL.

Figure 3 summarizes the obtained results. Figure 3a, 3b and 3c respectively show the lodscore curves corresponding to  $\log_{10} (H_2/H_0)$ ,  $\log_{10} (H_3/H_0)$  and  $\log_{10} (H_2/H_1)$ . It can be seen that very significant lodscores are obtained when testing for the presence of a paternally expressed QTL, while there is no evidence at all for the segregation of a QTL when studying the chromosomes transmitted by the sows. Also, the hypothesis of a paternally expressed QTL is significantly more likely ( $\log_{10} (H_2/H_1) > 3$ ) than the hypothesis of a "Mendelian" QTL

for all examined traits. The fact that the same tendency is observed for all traits indicates that it is likely the same imprinted gene that is responsible for the effects observed on the different traits. Table 2 reports the ML phenotypic means for the F2 offspring sorted by inherited paternal QTL allele. Note that when performing the analysis under a model of a mendelian QTL, the Piétrain and Large White QTL alleles appeared to behave in an additive fashion, the heterozygous genotype exhibiting a phenotypic mean corresponding exactly to the midpoint between the two homzygous genotypes. This is exactly what one would predict when dealing with an imprinted QTL as halve of the heterozygous offspring are expected to have inherited the P allele from their sire, the other halve the LW allele.

These data therefore confirmed our hypothesis of the involvement of an imprinted gene expressed exclusively from the paternal allele. The fact that the identified chromosomal segment coincides precisely with an imprinted domain documented in man and mice strongly implicates the orthologous region in pigs. At least seven imprinted genes mapping to this domain have been documented (*Igf2*, *Ins2*, *H19*, *Mash2*, *p57<sup>KIP2</sup>*, *KvLQTL1* and *TDAG51*) (ref. 15 and Andrew Feinberg, personal communication). Amongst these, only *Igf2* and *Ins2* are paternally expressed. While we cannot exclude that the observed QTL effect is due to an as of yet unidentified imprinted gene in this region, its reported effects on myogenesis *in vitro* and *in vivo*<sup>13</sup> strongly implicate *Igf2*. Particularly the muscular hypertrophy observed in transgenic mice overexpressing *Igf2* from a muscle specific promotor are in support of this hypothesis (Nadia Rosenthal, personal communication. Note that allelic variants of the *INS* VNTR have recently been shown to be associated

with size at birth in man<sup>16</sup>, and that the same VNTR has been shown to affect the level of *Igf2* expression<sup>17</sup>.

The observation of the same QTL effect in a Large White x Wild Boar intercross indicates the existence of a series of at least three distinct functional alleles. Moreover, preliminary evidence based on marker assisted segregation analysis points towards residual segregation at this locus within the Piétrain population (data not shown). The occurrence of an allelic series might be invaluable in identifying the causal polymorphisms which - based on the quantitative nature of the observed effect - are unlikely to be gross gene alterations but rather subtle regulatory mutations.

The effects of the identified QTL on muscle mass and fat deposition are truly major, being of the same magnitude of those reported for the CRC locus<sup>6,7</sup> though apparently without the associated deleterious effects on meat quality. We estimate that both loci jointly explain close to 50% of the Piétrain versus Large White breed difference for muscularity and leanness. Understanding the parent-of-origin effect characterizing this locus will allow for its optimal use in breeding programs. Indeed, today half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit the unfavourable Large White allele causing considerable loss.

The QTL described in this work is the second example of a gene affecting muscle development in livestock species that exhibits a non-mendelian inheritance pattern. Indeed, we have previously shown that the callipyge locus (related to the qualitative trait wherein muscles are doubled) is characterized by polar overdominance in which only the heterozygous individuals that inherit the CLPG mutation from their sire express the double-muscling phenotype<sup>5</sup>. This

demonstrates that parent-of-origin effects affecting genes underlying production traits in livestock might be relatively common.

### 5 Example 3:

Generating a reference sequence of IGF2 and flanking loci in the pig.

- 10 The invention provides an imprinted QTL with major effect on muscle mass mapping to the IGF2 locus in the pig, and use of the QTL as tool in marker assisted selection. To fine tune this tool for marker assisted selection, as well as to further identify a causal mutation, we have further generated  
15 a reference sequence encompassing the entire porcine IGF2 sequence as well as that from flanking genes.

- To achieve this, we screened a porcine BAC library with IGF2 probes and identified two BACs. BAC-PIGF2-1 proved to  
20 contain the INS and IGF2 genes, while BAC-PIGF2-2 proved to contain the IGF2 and H19 genes. The NotI map as well as the relative position of the two BACs is shown in Figure 5. BAC-PIGF2-1 was shotgun sequenced using standard procedures and automatic sequencers. The resulting sequences were assembled  
25 using standard software yielding a total of 115 contigs. The corresponding sequences are reported in figure 6. Similarity searches were performed between the porcine contigs and the orthologous sequences in human. Significant homologies were detected for 18 contigs and are reported in Figure 7.

30

For BAC-PIGF2-2, the 24 Kb NotI fragment not present in BAC-PIGF2-1 was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers. Resulting

sequences were assembled using the Phred-Phrap-Consed program suit, yielding seven distinct contigs (figure 8). The contig sequences were aligned with the corresponding orthologous human sequences using the compare and dotplot programs of the GCG suite. Figure 9 summarizes the corresponding results.

Example 4: Identification of DNA sequence polymorphisms in the IGF2 and flanking loci.

10 Based on the reference sequence obtained as described in Example 1, we resequenced part of the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals, allowing identification of DNA sequence polymorphisms such as reported in figure 10.

15



## Legends to the figures

Fig. 1: Test statistic curves obtained in QTL analyses of  
5 chromosome 2 in a Wild Boar/Large White intercross. The graph  
plots the F ratio testing the hypothesis of a single QTL at a  
given position along the chromosome for the traits indicated.  
The marker map with the distances between markers in Kosambi  
10 centiMorgan is given on the X-axis. The horizontal lines  
represent genome-wise significant ( $P < 0.05$ ) and suggestive  
levels for the trait lean meat in ham; similar significance  
thresholds were obtained for the other traits.

Figure 2: Piétrain pig with characteristic muscular  
15 hypertrophy.

Figure 3: Lodscore curves obtained in a Piétrain x Large  
White intercross for six phenotypes measuring muscle mass and  
fat deposition on pig chromosome 2. The most likely positions  
20 of the *Igf2* and *MyoD* genes determined by linkage analysis  
with respect to the microsatellite marker map are shown.  $H_0$   
was defined as the null-hypothesis of no QTL,  $H_1$  as testing  
for the presence of a Mendelian QTL,  $H_2$  as testing for the  
presence of a paternally expressed QTL, and  $H_3$  as testing for  
25 the presence of a maternally expressed QTL. 3a:  $\log_{10}(H_1/H_0)$ ,  
3b:  $\log_{10}(H_2/H_0)$ , 3c:  $\log_{10}(H_3/H_0)$

Figure 4: A. Structure of the human *Igf2* gene according to  
ref. 17, with aligned porcine adult liver cDNA sequence as  
30 reported in ref. 16. The position of the nt241(G-A)  
transition and Swc9 microsatellite are shown. B. The  
corresponding markers were used to demonstrate the  
monoallelic (paternal) expression of *Igf2* in skeletal muscle

and liver of 10-week old fetuses. PCR amplification of the *nt421(G-A)* polymorphism and *Swc9* microsatellite from genomic DNA clearly shows the heterozygosity of the fetus, while only the paternal allele is detected in liver cDNA (*nt421(G-A)* and *Swc9*) and muscle cDNA (*Swc9*). The absence of RT-PCR product for *nt421(G-A)* from in fetal muscle points towards the absence of mRNA including exon 2 in this tissue. Parental origin of the foetal alleles was determined from the genotypes of sire and dam (data not shown).

10

Figure 5: A NotI restriction map showing the relative position of BAC-PIGF2-1 (comprising INS and IGF2 genes), and BAC-PIGF2-2 (comprising IGF2 and H19 genes).

15 Figure 6: Nucleic acid sequences of contig 1 to contig 115 derived from BAC-PIGF2-1 which was shotgun sequenced using standard procedures and automatic sequencers.

Figure 7: Similarity between porcine contigs of figure 6 and orthologous sequences in human.

20

Figure 8 Nucleic acid sequences of contig 1 to contig 7 derived from BAC-PIGF2-2, (the 24 Kb NotI fragment not present in BAC-PIGF2-1) which was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers.

25

Figure 9: Similarity between porcine contigs of figure 8 and orthologous sequences in human.

30

Figure 10: DNA sequence polymorphisms in the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals.

REFERENCES

Literature cited with example 1

1. Andersson, L. et al. Genetic mapping of quantitative  
5 trait loci for growth and fatness in pigs. *Science* 263, 1771-  
1774 (1994).
2. Knott, S.A. et al. Multiple marker mapping of  
quantitative trait loci in a cross between outbred wild boar  
and Large White pigs. *Genetics* 149, 1069-1080 (1998).
- 10 3. Edfors-Lilja, I. et al. Mapping quantitative trait loci  
for immune capacity in the pig. *Journal of Immunology* 161,  
829-835 (1998).
4. Andersson-Eklund, L. et al. Mapping quantitative trait  
loci for carcass and meat quality traits in a wild boar x  
15 Large White intercross. *Journal of Animal Science* 76, 694-700  
(1998).
5. Fronicke, L., Chowdhary, B.P., Scherthan, H. &  
Gustavsson, I. A comparative map of the porcine and human  
genomes demonstrates ZOO-FISH and gene mapping-based  
20 chromosomal homologies. *Mamm Genome* 7, 285-90 (1996).
6. Alexander, L.J. et al. Physical assignments of 68  
porcine cosmids and lambda clones containing microsatellites.  
*Mammalian Genome* 7, 368-372 (1996).
7. Rohrer, G.A. et al. A comprehensive map of the porcine  
25 genome. *Genome Research* 6, 371-391 (1996).
8. Marklund, L. et al. A comprehensive linkage map of the  
pig based on a wild pig-Large White intercross. *Anim Genet*  
27, 255-69 (1996).
9. Marklund, L., Nyström, P.E., Stern, S., Andersson-  
30 Eklund, L. & Andersson, L. Quantitative trait loci for

fatness and growth on pig chromosome 4. *Heredity* In press(1998).

10. Ohlsson, R., Hedborg, F., Holmgren, L., Walsh, C. & Ekstrom, T.J. Overlapping patterns of IGF2 and H19 expression  
5 during human development: biallelic IGF2 expression correlates with a lack of H19 expression. *Development* 120, 361-368 (1994).
11. Ekström, T.J., Cui, H., Li, X. & Ohlsson, R. Promoter-specific IGF2 imprinting status and its plasticity during  
10 human liver development. *Development* 121, 309-316 (1995).
12. Hemberger, M. et al. H19 and Igf2 are expressed and differentially imprinted in neuroectoderm-derived cells in the mouse brain. *Dev. Genes Evol.* 208, 393-402 (1998).
13. Dunger, D.B. et al. Association of the INS VNTR with  
15 size at birth. *Nature Genetics* 19, 98-100 (1998).
14. DeChiara, T.M., Robertson, E.J. & Efstratiadis, A. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* 64, 849-859 (1991).
15. Sun, F.L., Dean, W.L., Kelsey, G., Allen, N.D. & Reik, W. Transactivation of Igf2 in a mouse model of Beckwith-Wiedemann syndrome. *Nature* 389, 809-815 (1997).
16. Davies, J.L. et al. A genome-wide search for human type 1 diabetes susceptibility genes. *Nature* 371, 130-136 (1994).
17. O'Dell, S.D. et al. ApaI polymorphism in insulin-like  
25 growth factor II (IGF2) gene and weight in middle-aged males. *International Journal of Obesity* 21, 822-825 (1997).
18. Falconer, D.S. & Mackay, T.F.C. *Introduction to Quantitative Genetics*, (Longman, England, 1996).
19. Hill, W.G. Rates of change in quantitative traits from  
30 fixation of new mutations. *Proc Natl Acad Sci U S A* 79, 142-145 (1982).

20. Marklund, S. et al. Molecular basis for the dominant white phenotype in the domestic pig. *Genome Research* 8, 826-833 (1998).
21. Kijas, J.M.H. et al. Melanocortin receptor 1 (*MC1R*) mutations and coat color in the pig. *Genetics* In press(1998).
22. Beechey, C.V. personal communication (1998).
23. Paquette, J., Giannoukakis, N., Polychronakos, C., Vafiadis, P. & Deal, C. The *INS* 5' variable number of tandem repeats is associated with *IGF2* expression in humans. *Journal of Biological Chemistry* 273, 14158-14164 (1998).
24. Sambrook, J., Fritsch, E.F. & Maniatis, T. *Molecular cloning : A laboratory manual.*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989).
25. Chowdhary, B.P., de la Sena, C., Harbitz, I., Eriksson, L. & Gustavsson, I. FISH on metaphase and interphase chromosomes demonstrates the physical order of the genes for GPI, CRC, and LIPE in pigs. *Cytogenetics Cell Genetics* 71, 175-178 (1995).
26. Green, P., Falls, K. & Crook, S. *Documentation for CRI-MAP, version 2.4.*, (Washington University School of Medicine, St Louise, MO, 1990).
27. Haley, C.S., Knott, S.A. & Elsen, J.M. Mapping quantitative trait loci in crosses between outbred lines using least squares. *Genetics* 136, 1195-1207 (1994).
28. Churchill, G.A. & Doerge, R.W. Empirical threshold values for quantitative trait mapping. *Genetics* 138, 963-971 (1994).
29. Anonymous. *SAS version 6.10*, (SAS Institute Inc., Cary, NC., 1990).

30

References used with example 2:

1. Fuji, J.; Otsu, K.; Zorzato, F.; Deleon, S.; Khanna, V.K.; Weiler, J.E. O'Brien, P.J.; MacLennan, D.H. (1991). Identification of a mutation in the porcine ryanodine receptor associated with malignant hyperthermia. *Science* 253: 448-451.
2. MacLennan, D.H. & Phillips, M.S. (1993). Malignant hyperthermia. *Science* 256:789-794.
3. Grobet, L.; Royo Martin, L.J.; Poncelet, D.; Pirottin, D.; Brouwers, B.; Riquet, J.; Schoeberlein, A.; Dunner, S.;  
10 Ménissier, F.; Massabanda, J.; Fries, R.; Hanset, R.; Georges, M. (1997) A deletion in the myostatin gene causes double-muscling in cattle. *Nature Genetics* 17:71-74.
4. Andersson, L.; Haley, C.S.; Ellegren, H.; Knott, S.A.; Johansson, M.; Andersson, K.; Andersson-Eklund, L.; Edfors-  
15 Lilja, I.; Fredholm, M.; Hansson, I.; Håkansson, J.; Lundström, K. (1994). Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science* 263:1771-1774.
5. Cockett, N.; Jackson, S.; Shaw, T.; Farnir, F.; Berghmans, S.; Snowden, G.; Nielsen, D.; Georges, M. (1996). Polar  
20 overdominance at the ovine callipyge locus. *Science* 273:236-238
6. Hanset, R.; Dasnois, C.; Scalais, S.; Michaux, C.; Grobet, L. (1995). Genotypes at the locus for halothane sensitivity and performance in a Piétrain x Large White F2. *Genet. Sel. Evol.* 27: 63-76.
7. Hanset, R.; Dasnois, C.; Scalais, S.; Michaux, C.; Grobet, L. (1995). Introgression into the Piétrain genome of the normal allele at the locus for halothane sensitivity. *Genet. Sel. Evol.* 27: 77-88.
8. Olivier, L.; Lauvergne, J.J. (1967). A study of the  
30 inheritance of the muscular hypertrophy of the Piétrain pig: preliminary results. *Annales de Médecine Vétérinaire* 111: 104-109.

9. Rettenberger, G.; Klett, C.; Zechner, U.; Kunz, J.; Vogel, W.; Hameister, H. (1995). Visualisation of the conservation of synteny between humans and pigs by heterologous chromosome painting. *Genomics* 26: 372-378.
- 5 10. Goureau, A.; Yerle, M.; Schmitz, A.; Riquet, J.; Milan, D.; Pinton, P.; Frelat, G.; Gellin, J. (1996). Human and porcine correspondence of chromosome segments using bidirectional chromosome painting. *Genomics* 36:252-262.
11. Yun, K.; Wold, B. (1996). Skeletal muscle determination and differentiation: story of a core regulatory network and its context. *Current Opinion in Cell Biology* 8:877-889.
- 10 12. Knoll, A.; Nebola, M.; Dvorak, J.; Cepica, S. (1997). Detection of a DdeI PCR RFLP within intron 1 of the porcine MYOD1(MYF3) locus. *Animal Genetics* 28, 308-322.
- 15 13. Florini, J.R.; Ewton, D.Z.; McWade, F.J. (1995). IGFs, muscle growth, and myogenesis. *Diabetes Review* 3:73-92.
14. Catchpole, I.R.; Engström, W. (1990). Nucleotide sequence of a porcine insulin-like growth factor II cDNA. *Nucleic Acids Research* 18(21):6430.
- 20 15. Feil, R.; Moore, T.F.; Oswald, J.; Walter, J.; Sun, F.; Reik, W. (1997). The imprinted insulin like growth factor 2 gene. Pp70 In *Genomic Imprinting*. Eds. Reik & Surani. IRL Press at Oxford University Press.
16. Dunger, D.B.; Ong, K.K.L.; Huxtable, S.J.; Sherriff, A.; Woods, K.A.; Ahmed, M.L.; Golding, J.; Pembrey, M.E.; Ring, S.; the ALSPAC study team, Bennett, S.T.; Todd, J.A. (1998). Association of the INS VNTR with size at birth. *Nature Genetics* 19: 98-100.
- 25 17. Paquette J, Giannoukakis N, Polychronakos C, Vafiadis P, Deal C. (1998) The INS 5' variable number of tandem repeats is associated with IGF2 expression in humans. *J. Biol Chem* 273(23):14158-14164
- 30

18. Andersson-Eklund, L.; Marklund, L.; Lundström, K.; Haley, C.S.; Andersson, K.; Hansson, I.; Moller, M.; Andersson, L. (1998). Mapping Quantitative Trait Loci for carcass and meat quality traits in a Wild Boar x Large White intercross. *J. Anim. Sci.* 76:694-700.
19. Rohrer, G.A.; Alexander, L.J.; Hu, Z.; Keele, J.W.; Smith, T.P.; Beattie, C.W. (1996). A comprehensive map of the porcine genome. *Genome Research*, in the press.
20. Georges, M.; Nielsen, D.; Mackinnon, M.; Mishra, A.; Okimoto, R.; Pasquino, A.T.; Sargeant, L.S.; Sorensen, A.; Steele, M.R.; Zhao, X.; Womack, J.E.; Hoeschele, I. (1995). Mapping quantitative trait loci controlling milk production by exploiting progeny testing. *Genetics* 139: 907-920.
21. Baron, H.; Fung, S.; Aydin, A.; Bahring, S.; Luft, F.C.; Schuster, H. (1996). Oligonucleotide ligation assay (OLA) for the diagnosis of familial hypercholesterolemia. *Nat. Biotechnol.* 14(10):1279-1282.
22. Lander, E.; Green, P. (1987) Construction of multilocus genetic linkage maps in humans. *Proceedings of National Academy of Science (USA)* 84: 2363-2367.
23. Lalouel, J.M. (1983). Optimization of functions. *Contrib. Epidemiol.Biostat.* 4:235-259.
24. Lander, E.S. & Botstein, D. (1989). Mapping mendelian factots underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185-199.
25. Spelman RL, Coppieters W, Karim L, van Arendonk JAM, Bovenhuis H (1996) Quantitative trait loci analysis for five milk production traits on chromosome six in the dutch Holstein-Friesian population. *Genetics* 144:1799-1808.
26. Chirgwin, J.M.; Przybyla, A.E.; MacDonald, R.J.; Rutter, W.J. (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18:5294-5299



Table 1 Summary of QTL analysis for pig chromosome 2 in a Wild Boar/Large White intercross<sup>1</sup>

Trait	F ratio <sup>2</sup>	Map position <sup>3</sup>	Percent of F <sub>2</sub> variance <sup>4</sup>	Least squares means <sup>5</sup>						
	QTL	Imprinting		WP/WM	WP/LM	LP/WM				
<i>LP/LM</i>										
<u>Body composition traits</u>										
5	Lean meat in ham, %	24.4***	19.1***	0	30.6	63.6 <sup>a</sup>	64.2 <sup>a</sup>	66.4 <sup>b</sup>	67.3 <sup>b</sup>	
	Lean meat mass in ham, kg	18.1***	16.8***	1	24.3	4.69 <sup>a</sup>	4.72 <sup>a</sup>	4.94 <sup>b</sup>	5.02 <sup>b</sup>	
	Lean meat + bone in back, %	12.2**	9.6**	0	17.4	66.3 <sup>a</sup>	66.7 <sup>a</sup>	69.3 <sup>b</sup>	70.8 <sup>b</sup>	
	Longissimus muscle area, cm <sup>2</sup>	10.3**	4.8*	1	15.4	31.9 <sup>a</sup>	33.0 <sup>a</sup>	34.5 <sup>b</sup>	35.2 <sup>b</sup>	
<u>Fatness traits</u>										
15	Average back fat depth, mm	7.1*	8.7**	0	10.4	27.2 <sup>a</sup>	27.7 <sup>a</sup>	25.5 <sup>b</sup>	24.7 <sup>b</sup>	
<u>Weight of internal organs</u>										
	Heart, gram	9.7**	11.4***	0	14.4	226 <sup>a</sup>	225 <sup>a</sup>	238 <sup>b</sup>	244 <sup>b</sup>	
<u>Meat quality traits</u>										
20	Reflectance value, EEL	5.7	6.1*	1	8.1	18.6 <sup>a</sup>	18.4 <sup>a</sup>	21.8 <sup>b</sup>	19.7 <sup>a</sup>	

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001

\**P*<0.05; \*\**P*<0.01; \*\*\**P*<0.001

**Table 1, continued**

- <sup>1</sup>Only the traits for which the QTL peak was in the *IGF2* region (0-10 cM) and the test statistic reached the nominal significance threshold of  $F=3.9$  are included.
- <sup>2</sup>"QTL" is the test statistic for the presence of a QTL under a genetic model with additive, dominance, and imprinting effects (3 d.f.) while "Imprinting" is the test statistic for the presence of an imprinting effect (1 d.f.), both obtained at the position of the QTL peak. Genome-wise significance thresholds, estimated by permutation, were used for the QTL test while nominal significance thresholds were used for the Imprinting test.
- <sup>3</sup>In cM from the distal end of 2p; *IGF2* is located at 0.3 cM.
- <sup>4</sup>The reduction in the residual variance of the  $F_2$  population effected by inclusion of an imprinted QTL at the given position.
- <sup>5</sup>Means and standard errors estimated at the *IGF2* locus by classifying the genotypes according to the population and parent of origin of each allele. *W* and *L* represent alleles derived from the Wild Boar and Large White founders, respectively; superscript *P* and *M* represent a paternal and maternal origin, respectively. Figures with different letters (superscript a or b) are significantly different at least at the 5% level, most of them are different at the 1% or 0.1% level.

Table 2 Maximum likelihood phenotypic means for the different F2 genotypes estimated under (i) a model of a mendelian QTL, and (ii) a model assuming an imprinted QTL.

Traits	Mendelian QTL				Imprinted QTL		
	$\mu_{LW/LW}$	$\mu_{LW/P}$	$\mu_{P/P}$	R	$\mu_{PAT/LW}$	$\mu_{PAT/P}$	R
BFT (cm)	2.98	2.84	2.64	0.27	2.94	2.70	0.27
% ham	21.10	21.56	22.15	0.83	21.23	21.9 5	0.83
% loin	24.96	25.53	26.46	0.91	25.12	26.1 4	0.93
% lean cuts	65.02	65.96	67.60	1.65	65.23	67.0 5	1.67
% backfat	6.56	6.02	5.33	0.85	6.43	5.56	0.85
% fat cuts	28.92	27.68	26.66	1.46	28.54	26.9 9	1.49

CLAIMS

1. A method for selecting a domestic animal for having desired genotypic properties comprising testing said animal for the presence of a parentally imprinted quantitative trait locus (QTL).
- 5 2. A method according to claim 1 further comprising testing a nucleic acid sample from said animal for the presence of a parentally imprinted quantitative trait locus (QTL).
3. A method according to claim 1 or 2 wherein in the pig said QTL is located at chromosome 2.
- 10 4. A method according to claim 2 or 3 wherein said QTL is mapping at around position 2p1.7.
5. A method according to claim 1 to 4 wherein said QTL is related to the potential muscle mass and/or fat deposition of said animal.
- 15 6. A method according to claim 5 wherein said QTL comprises at least a part of an insulin-like growth factor-2 (IGF2) gene.
7. A method according to anyone of claims 1 to 6 wherein in the pig said QTL comprises a marker characterised as nt241(G-
- 20 A) or as Swc9, as identified in figure 4.
8. A method according to anyone of claims 1-7 wherein a paternal allele of said QTL is predominantly expressed in said animal.
9. A method according to anyone of claims 1-7 wherein a
- 25 maternal allele of said QTL is predominantly expressed in said animal.
10. An isolated and/or recombinant nucleic acid comprising a parentally imprinted quantitative trait locus (QTL) or functional fragment derived thereof.
- 30 11. An isolated and/or recombinant nucleic acid comprising a synthetic parentally imprinted quantitative trait locus (QTL)

derived from at least one chromosome or functional fragment derived thereof.

12. A nucleic acid according to claim 10 or 11 at least partly derived from a *Sus scrofa* chromosome.

5 13. A nucleic acid according to claim 12 wherein said nucleic acid is at least partly derived from a *Sus scrofa* chromosome 2, preferably from a region mapping at around position 2p1.7.

14. A nucleic acid according to any one of claims 10 to 13 wherein said QTL is related to the potential muscle mass  
10 and/or fat deposition of said animal.

15. A nucleic acid according to any one of claims 10 to 14 wherein said QTL comprises at least a part of a insulin-like growth factor-2 (IGF2) gene.

16. A nucleic acid according to anyone of claims 10 to 15  
15 wherein a paternal allele of said QTL is capable of being predominantly expressed.

17. A nucleic acid according to anyone of claims 10 to 16 wherein a maternal allele of said QTL is capable of being predominantly expressed.

20 18. Use of a nucleic acid or fragment derived thereof according to claim 10 in a method according to anyone of claims 1-9.

19. Use according to claim 18 to select a breeding animal or animal destined for slaughter for having desired genotypic or  
25 potential phenotypic properties.

20. Use according to claim 19 wherein said properties are related to muscle mass and/or fat deposition.

21. An animal such as pig selected by a use according to claim 18 to 20.

30 22. A animal according to claim 21 characterised in being homozygous for an allele at a paternally imprinted QTL, preferably located at a *Sus scrofa* chromosome 2 mapping at around position 2p1.7.

23. An animal according to claim 21 or 22 wherein said QTL is  
35 related to the potential muscle mass and/or fat deposition of

said pig and/or wherein said QTL comprises at least a part of a insulin-like growth factor-2 (IGF2) allele.

24. A transgenic animal comprising a nucleic acid according to anyone of claims 11 to 16.

5 25. An animal according to anyone of claims 21-24 which is a male.

26. Sperm or an embryo derived from an animal according to anyone of claims 21-25.

27. Use of a sperm or an embryo according to claim 26 in  
10 breeding animals destined for slaughter.

FIGURE 1

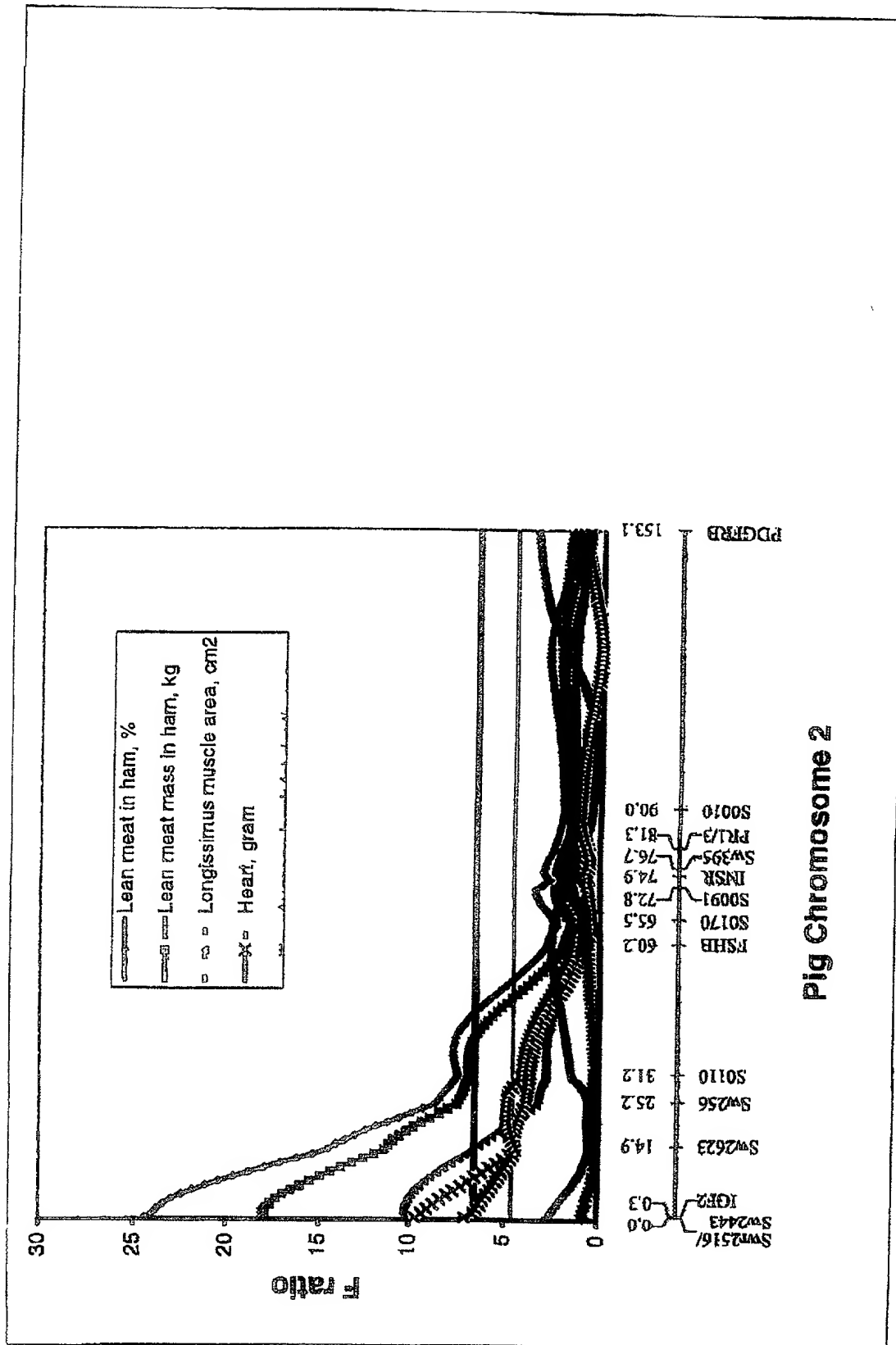


FIGURE 2





FIGURE 3A

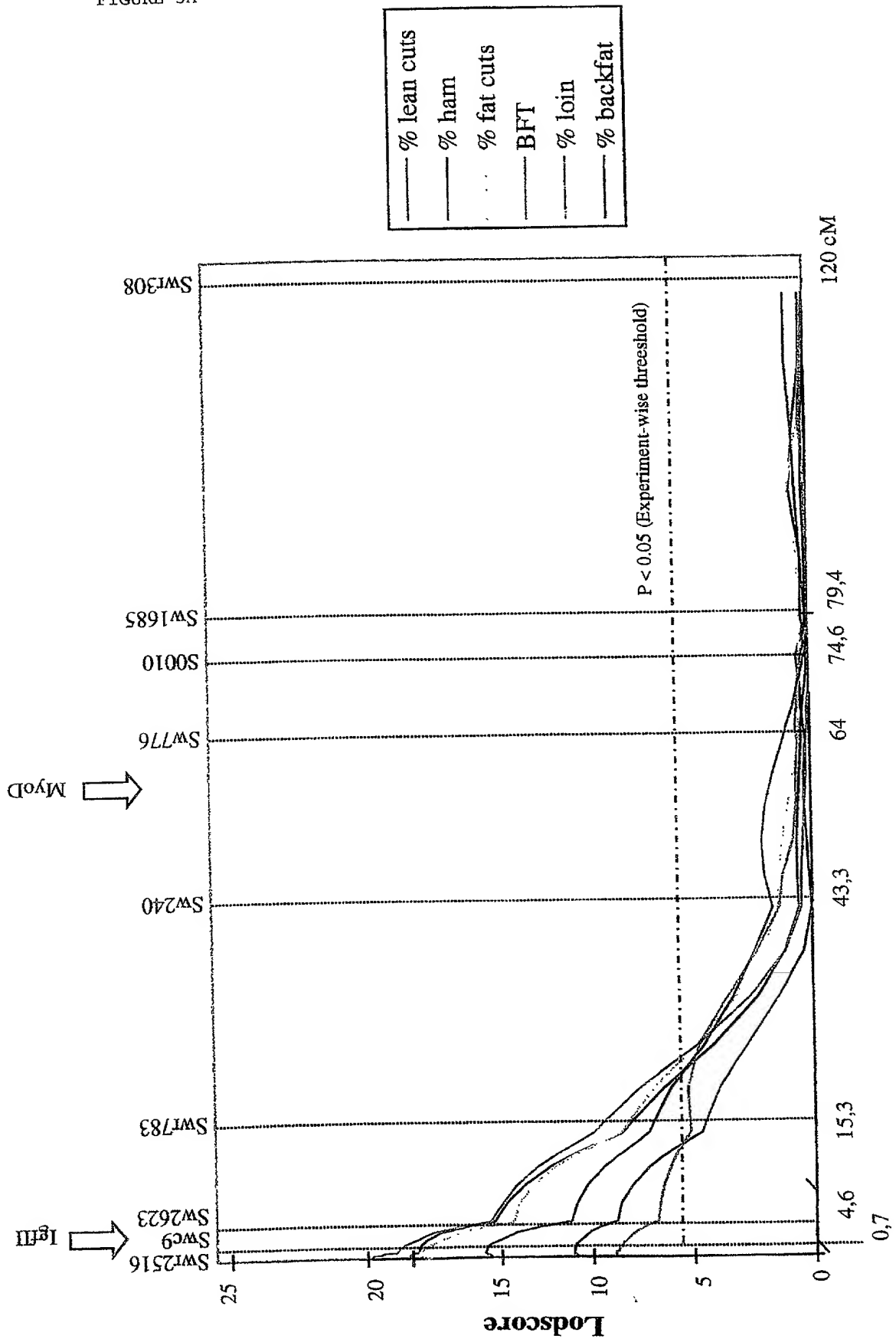


FIGURE 3B

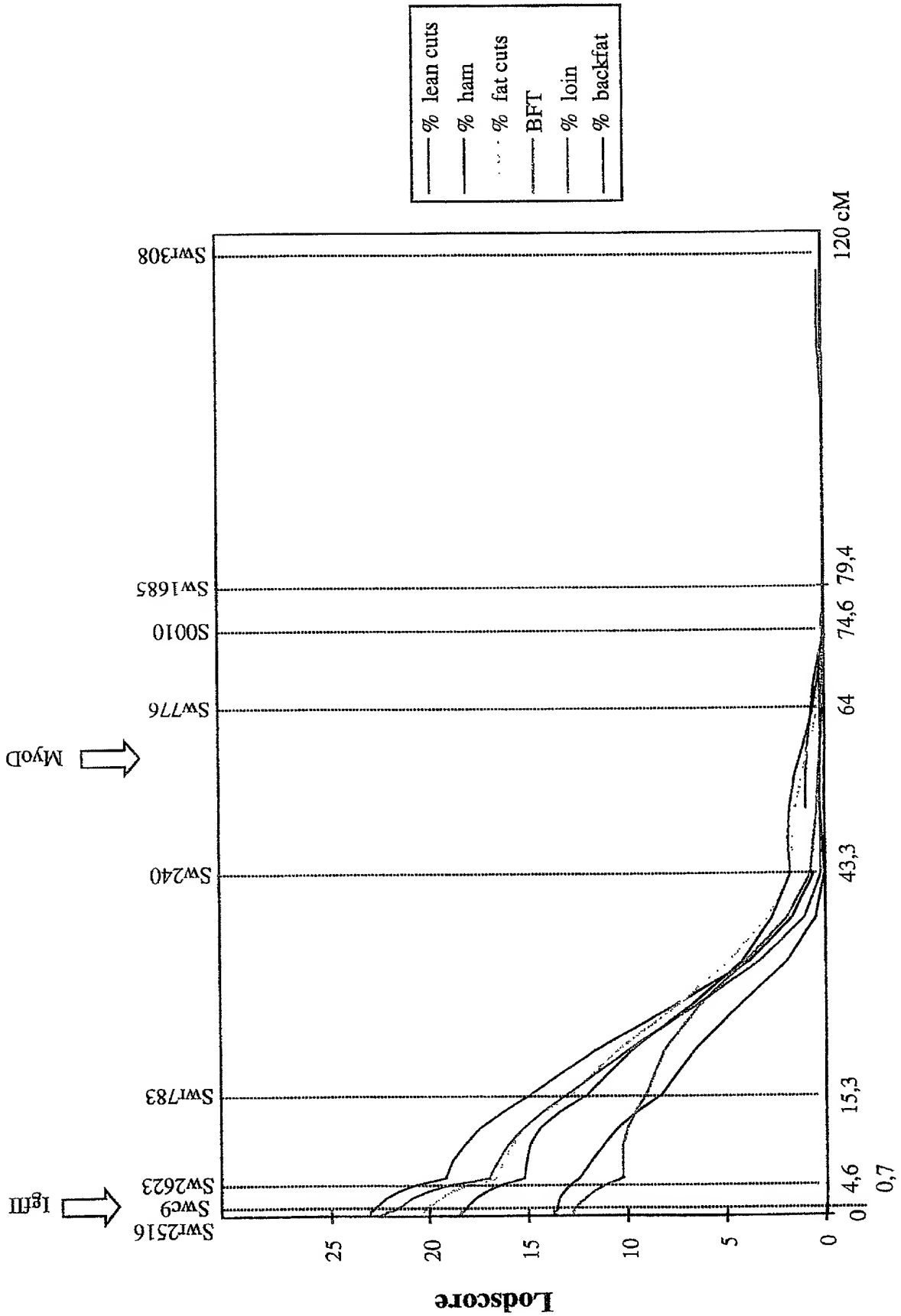


FIGURE 3C

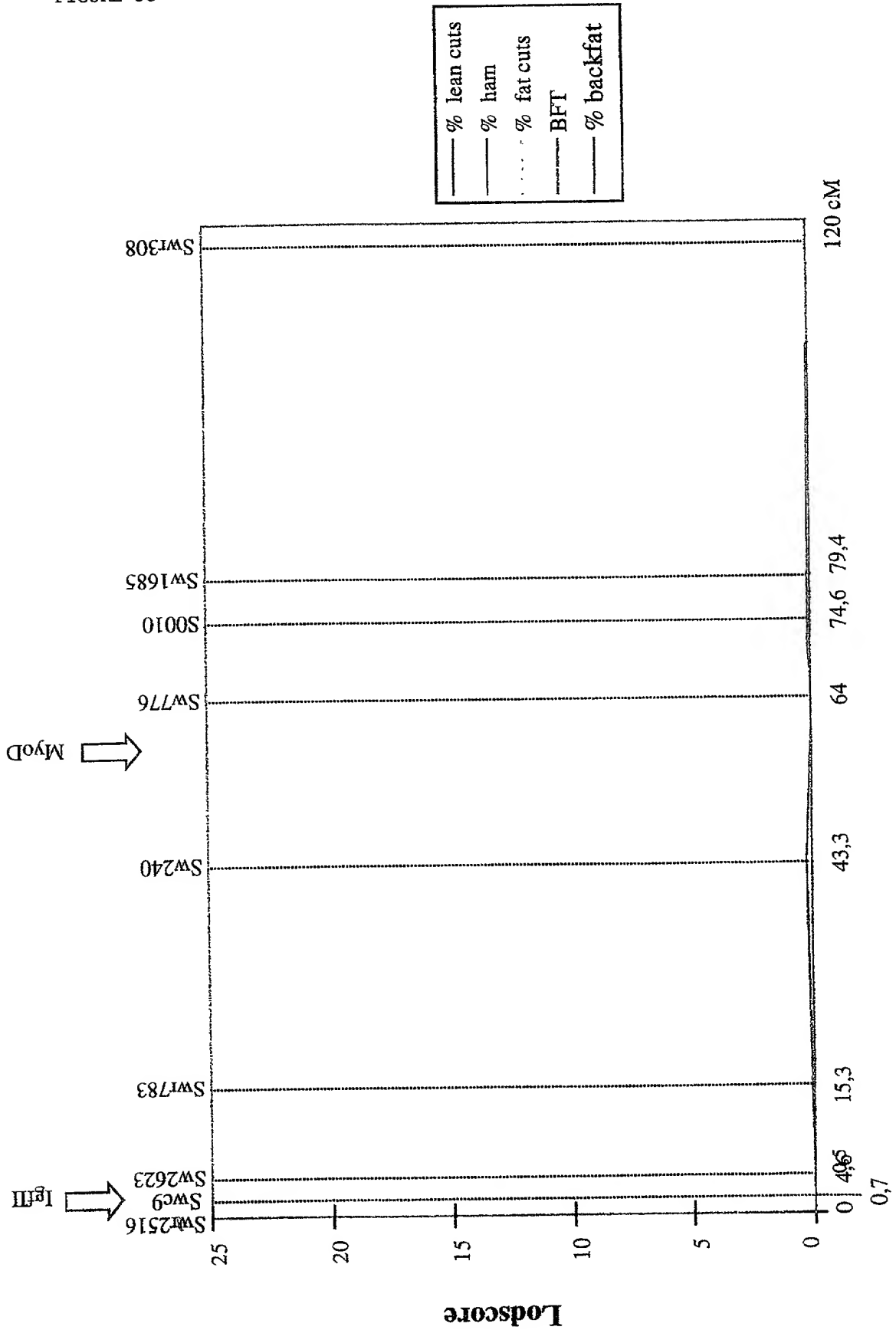


FIGURE 4

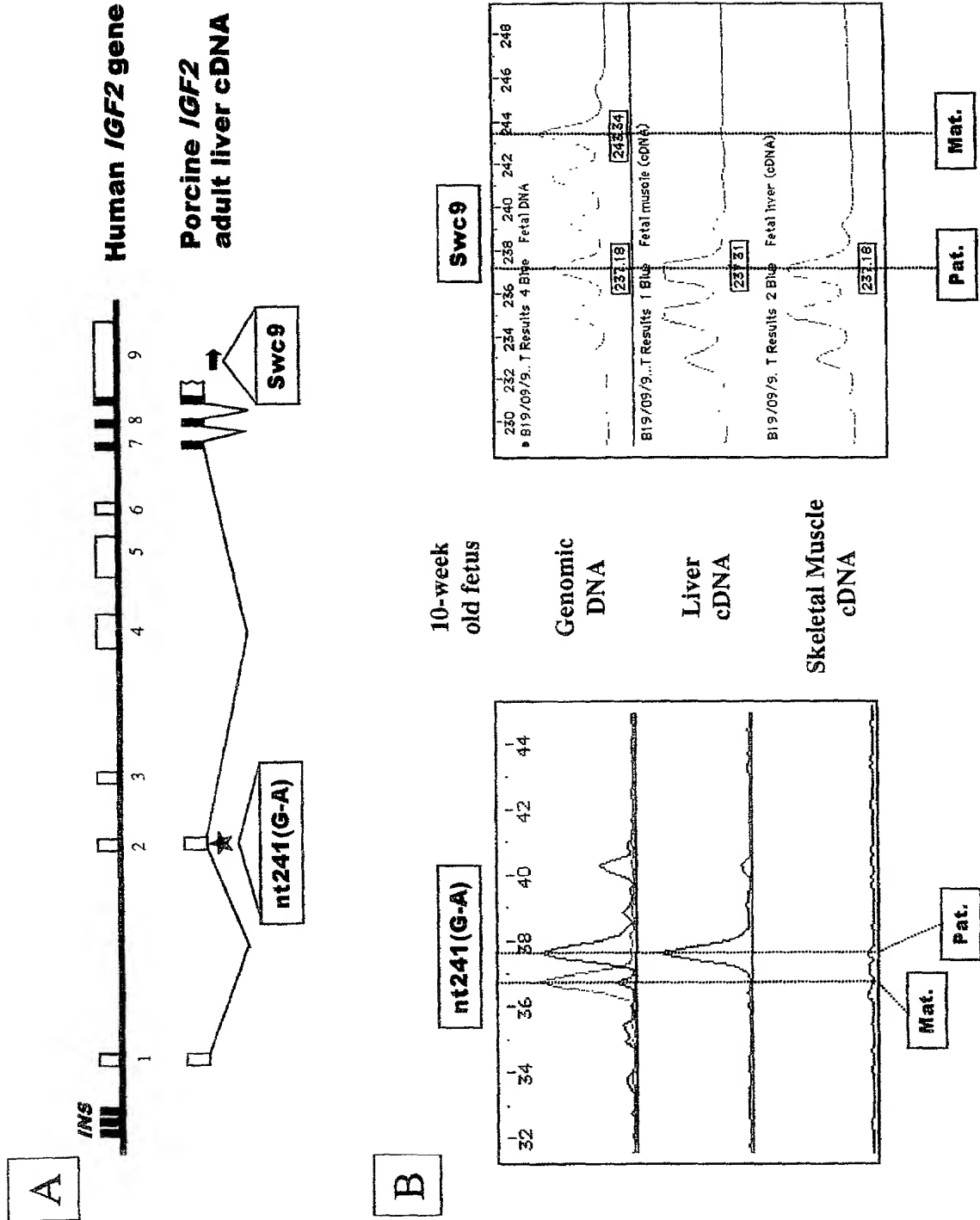


FIGURE 5

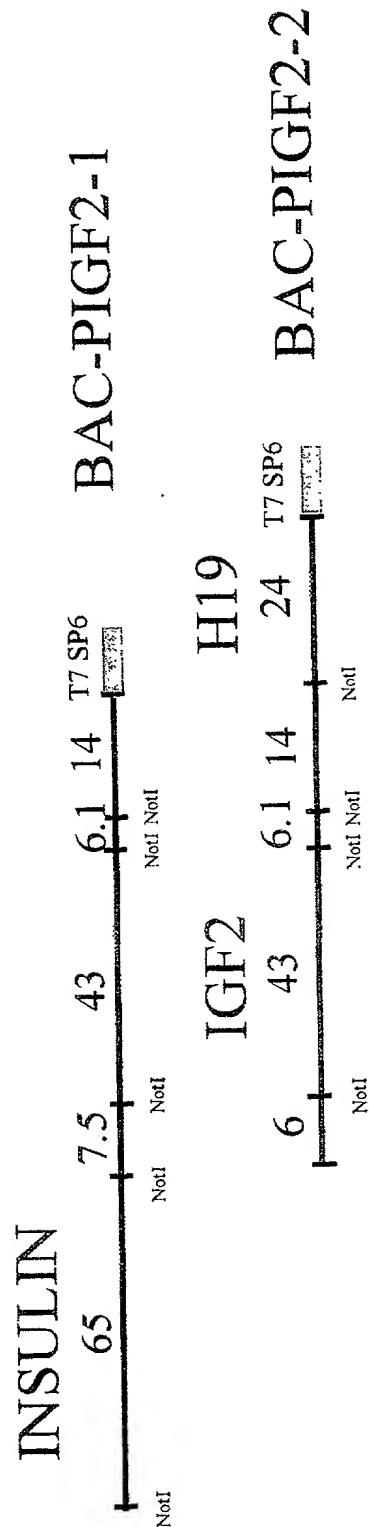


FIGURE 6

## Contig 1 (500 bp)

GGGTGGGCAGCTTCCCTCCAGACCGCAGGAGGCCCAAGTTCCCTGGCCCTGCCACCCAGGGCCAGCTGAAGC  
 AGGTACAGAGACACCCGCTCCTGTCCCTCCTGTACCTAACCCAACAGGCCGGGGCCAGGGACACAGGCCACA  
 TGGCATCTCCCCCATGCCCCTGCCCAAGGCGCCAGCAGGTGAGGCTGGAGCAGAGTCTGGGTCCCTGCGGG  
 CCAGACCGAGGGCAGGACAGCTGGGCATCTGTCTCACAGTCCCCGCGCTTTGTCTGGGAGGCGGCAGAGCCTC  
 ATCCAAGACGCCCCGAAGGAACGGGAGAAGGCGGAGGCGCGGCTGCCGCGTCCGAGCCCGGGGAGGCCCTGG  
 AAGTGGGGGCCCTTGGCCAGCGGACGGGAAGGCCCTGCTGAACCTGCTCTTACCCTGAGGCCACCAAGCC  
 CCCCTCGCTGTTCCGGTCCCTGAAAAATTCTAGGTGAGGGGGCGGGCCAGGGCTCCCCGGG

## Contig 2 (943 bp)

TGCTCCTCACACCCCGGGCGGGGCTGCTCTTGGGGCCATCCTCCCCATGGGGCCAGCACCCACTCTGGCCTTC  
 ACACCTGCCGTCTTCTGGGAAGTCCCTCTGGTTCCCAAGGAAAGTTTCTGAGCTGGACAAGTGCCACCACCTGG  
 TCACCAAGTTTCGATCCTGAGCTGGACCTGGACCACCCGGTGAGCCGGTGCCTCCCCCGGCCGCCATGTC  
 TCCCATCCCCAGGGGTGTCCCCACACTCAGGGCCGGGACTGGGCGTGAACCCCGGGTGGGACGGATGTTGGC  
 CTGCTGTGTGGCTCCTGGCGGAACAGAGAGGCTGGCTGGGTGCCACCCCGAGGCCCGCCGATGACACGG  
 GCCGCGTCTGGGCTGGGCGGGCAGGGCGGCCAGGC  
 AGGGCAGCCTCCGATGGCTCCCCGGGTGTACCAGGGCTTCTCGGACCAGTTGTACCGCCAGCGCAGGAAGC  
 TGATTGCCCAGATCGCCTTCCAGTACAGGCAGTAAGTCCCTCCAGGGCCTCAGCCTGGGGGCCAGACCTCAG  
 CCTGGGCCTCACGCCAGACCTGGGGGTGGAGGGAAGGGAGGTTGTCTTTGTACCAACGCCACCACCTTCACT  
 GTACCATGGTACCGACTCTGGGTCCCCAAATCACAGCTGAGGAACTGGGGCACAGAGTGGTTAAGCATCT  
 TGCTGAAGCCACACAGCTGGCGGAGCATTGGCCCCGGCCCCCTCTGCGGCTCCACACGTGCTCCCTGAGGG  
 CCCCCGACTGACAGCTGTCCCCCTCCTCAGAGTG  
 ACCCTATTCCCGCTGGAGTACACAGCCGAGGAGATTGCCACCTGGTGAGGCCCTGTGACAGCGGCTGGGAG  
 GGGCGGAGTGGGGGAAGGGACAGGAAGACCTCAGAATTCCCGCTGGAACGTGGTGGCCTCTATCATGA

## Contig 3 (1500 bp)

GGGGAGGGGATGCTCAGACCCGCTCTGGGAAGAAGAGAGCCTCAGAAGAAATCCCTTCCCAAGGGTCACGCGG  
 TGGAGCCCAGGGGCCCGCTAGGGGCCGATTCCACAGCTCGTCTGCCACCTGCTGGCGCTCCAGGAACTGC  
 GGAGCGGTGGGGGCCCTGGATGGGTCCGGCAGTGGGCTCGCAGGAGACCCCTGGAGGGGCTGCGGACACCCC  
 AGCTGCCACTCACAAGGTGCCAAGCGGCGGTGGCAATGGGCTGAGCCTCTCCCCCTCCTCCTCCGAGGA  
 CATTGGCCTCGCATCCCTGGGGGTCTCGGACGAGGAAATTGAGAAGCTGTCCACGCTGGGTTTCTCCCCCTGC  
 AGGGCCTGGGTTCCAGCCAGGCCCTCCTGTCCAA  
 GGGGTGTCGTCTCAGCTGTGACCGCCCGGGAGCCTGGATCGGTTCTGCCTGGGTGGGCGGTGCCCGGCCA  
 CGGGCAGCAGGGGCAGCGGTGCGGGCCCGAGCCGTGTCTGAGCCCCCTTGCCGCTGTCCCCACAGCTGTAC  
 TGGTTACCGGTGGAGTTTGGGCTCTGCAACAGCAACGGCGAGGTGAAGGCCCTACGGGGCTGGGCTGTCTCT  
 CCTACCGGGAGCTCCTGGTGAGGCCTCCCCACGCGCTGGGGCCTGGGTCCCCGGGGAGGTGACCCCTGCGG  
 TGCCTTGTGGATTCCAGCTCTCGGGAGGCTGGAGCGAGGGGCTGCCCTCCTGGGGGACCAAGAAAGCTGGTC  
 TGGCCCCCTCTCCACACACCTGTGCCTGGGCCCTG  
 GGGGGACCCCTGCTGGGGGATGTGGGTGCACAGCCAGGGCCACCAGGGAGTCAGGACACGGGGCTCCCTTCCC  
 TCGGGTCCCTGAGACCCCTGGCCTCCCGCCAGCACTCCCTGTCCGAGGAGCCCGAGATCCGGGCCCTTCGACCC  
 CGACGCGCGCGCGTGCAGCCCTACCAGGACCAGACCTACCAGCCCGTCTACTTCTGTCTGAGAGTTTCAGT  
 GACGCCAAGGACAAGCTCAGGTGGGCGGGGCCCGGGGCCCAAACTGGAGGATCCAGCTGCAGCCCCGCC  
 TATGAGCCATTTCACAGCAGAGGGAGCTGCTGCGGACCCACCGTCACAACCCCCCTCCACAGCTGGAACC  
 CCAGAAAGCCTGCGGAGGGGGGACCTGCAGGGCTG  
 TGGCCAGGTCAAGGCCAGGTGAGGCCAGGCTTTAGGGGTGAAGTCTGACTTTGTAAAGAGGGGGTGCAGGGT  
 CCTTCCAGCCTCCTCCCTCCGAGCAGCTGGGGCGGGGCGGGGTGCGATGAAGGCAGAGATGACGCAGCC  
 ACCGTTTACCCCTCAGGAGGCGCTCCTGTCCAGCCAGGCTCCTGTGTACAGGGGAACTGAGGCCCGAGG  
 TGTGTGTGGGGGGGTGATTCTCACACAAAGCTTAGGGACAGGGACATAACGGCCTCTCCAGGGCACACAG  
 TCTGGAGG

## Contig 4 (3024 bp)

TTAANTCCANGTTGGCCCCACAAGTTTTCCCCATTGAAAAGGGGCCAGTTAAGCCCCAACNCAATTAATTGG  
 AAGTTAGCTCCCTCATTAGGCTCCCCAGNCTTTACNCTTTATGTTCCGGTTTCGTATTTTGTGGGAATTGTA  
 GCGGATACAAATTTCTCTCAAGNAACCAGCTATGCCCATGATTACGCGGTACAGTAGTTTATCAGTCCCCCCC  
 CCCATGGGACAGCGAAGGGAACCAAGTATGTCGTGGGGCCGGGTCTAAAGGGGTACCACCAGGGAGGGGCAGG  
 GGCTCCAGGAGGCAGGGCCACTGAGCGGTACCTGGTGGGGGGAGGTGGTGGGGCCACACCAGGAGTCTGTG  
 CCCCCCTTCCCGCGTGGACATGAGAAGCAGGGGCCAGCCTGCGGGTCCCTGAGTTACGCGCCCCCCCC  
 CCCCCCCCCGAGCAGCCCCGGGTCTCAGCAGGCTGCTGTGCTGGGGCGGGGCGGCTTATGGRGCCGGGAG  
 CAGCCCCCCCCACGGCTTACAGCATCTCTGGGGCCTCAGGATGGACCGGGGTCTGCRGGCAGGTGTCTCTC  
 TCGCGCCCCCTTCCCTGGGCTATAACGTGGAAGATGCGGCCCAAGCCCGGKCGGTTTGGCCTTTGTCCCCAG  
 CCAGTGGGGACAGCCTGGCCCTCAGGCCGCTCGTTAAGACTCTAATGACCTCAAGGCCCCAGAGGCGCTGAT  
 GACCCACGGAGATGATCCCGAGGCTGGCAGCAGGGAAATGATCCAGAAAGTGCCACCTCAGCCCCAGCCA

FIGURE 6, CONTD.

TCTGCCACCCACCTGGAGGCCCTCAGGGGCCGGGCGCCGGGGGAGGCGCTATAAAGCCGGCCGGGGCCAGC  
CGCCCCAGCCCTCTGGGACCAGCTGTGTTCCAGGCCACCGGCAAGCAGGTCTGTCCCCCTGGGCTCCCGTC  
AGCTGGGTCTGGGCTGTCTGCTGGGGCCAGGGCATCTCGGCAGGAGGACGTGGGCTCCTCTCTCGGAGCCCT  
TGGGGGGTGGAGCTGGTGGGGGCTGCAGGTGCCCTGGCTGGCCTCAACGCCGCCCGTCCCCCAGGTCTCTAC  
CCCCGCCATGGCCCTGTGGACGCGCCTCCTGCCCTGTGGCCCTGTGGCCTCTGGGCGCCCGCCCGG  
CCAGGCCTTCGTGAACCAGCACCTGTGCGGCTCCACCTGGTGGAGGCGCTGTACCTGGTGTGCGGGGAGCGC  
GGCTTCTTCTACACGCCCAAGGCCCTCGGGAGGCGGAGAACCCTCAGGGTGAGCCGAGGGGGYGTCCCCGGA  
GCGGTGGGGGAGTTTTTAAGGAGGAAATTGGTAAAGTGACCAACTCCCTGGGAGCTGAGCCAGAGACACC  
CCTCCACGCCCCYGGTCCCGCTCGAGAAGCCCCCTTCCCTCCCTCCTCCCG  
AGGCGGCTCCAGGGAGGAATCTTACGGAGTCAAGGCCCGGGTGCCGCTGGTCTCCGAGTGACATGGCCGTGGT  
GTCCRTCTGCCGGCCACATGCCCGTGAGAGAWGCCCATCCCCCTGGGAGGGGGCCCGTGCCGGGAGGC  
GGCGGGAGGCCAGGACCGGTGGCTGTGCGGCTTCCACTCCAGGGTGGGCGGGGTGGGGGGTGGCTGTCTCT  
GTGTGACCGGCTCTCCCCGAGCAGGTGCGGTGGAGCTGGGCGGAGGCTGGGCGGCTGCAGGCCCTGGCGC  
TGGAGGGGCCCGCAGAAGCGTGGCATCGTGGAGCAGTGTGTCACCAGCATCTGTTCCTCTACCAGCTGGA  
GAACTACTGCAACTAGGCCGCCCTGAGGGCGCTGTGCTCCCCGACCCCAAAACCAATAAAGTCTTGAA  
TGAGCCCGGCCGAGTCTGTGGTCTGTGTGGCCTGGGGCGGGGCCCTGGTGGGGAGGGGCCAGAAAGCTGT  
GGGGGGCTGCTGCGACCCCTCTGTGCTCTGCGCACATCGGCTGCTCTAAGCTTCTCCACATGCATCGGGT  
GCCCCAGGCACATGGGCACCGGGGGACAGGGGCCAGGGCAGGGCCCTTCAATGTGGCGAGCTCTGGTTTTT  
AGGGCTCCAGACACCCCTCCTGGGTGCCACTGTGCACAGGGTCACTCTGAGGGTACAGGGCACCCACC  
AGACTGCTCTTGGGCACAAATAGCCAGGGGCTTCTTGGGCTGGCTGCRGTCTGGGAGGTACAGAGTGA  
CCCCCGGGACCAAGACCTGGCCAGCCTGCCAGTGCAGGCCAAACCAATCTGCACCTTTGCTGAAGGTTT  
CACCCGGGCCAGCACTGGGGGCGGCCGGGCTAGAGCTGGGCGCCCGGGCCAGGGACTGCACACCCGCCAG  
AGGTGGGCTGAGGGGTGGCAGCAGGCTCTCCGCTGGGACCCAGCCAGCTGGGCGAGTCACTCTCAACAG  
AGGCTCTACCTGTGTCTCCCTCCCCACGGCCACACAGACACCCCTGGGGAGAGTACAGGCCCCAGCA  
GGCCCCGCCCTGGAGAGGAGGCCAGGGCTGGGCGAGGCGGGTGGCCGGCCGACACTGGACCCGGAAGGGGG  
TAGGCGGCTGGGATGAGTGGCGAGCTGTCCATGGGAGCACCAGCGGCCCCATTGGCACCACTACAGGCAGGG  
GCACCTGCAGCAGCTGAGGTACGTGGGGTCCCCGGAAGTGGTGGTGTCCGGCTGCCCTCTGGGAGGCAGCGG  
CTGAGCTTGTGGTCTGCCAACCAGGGAGACCCGTGACCACCTGCTGCTTCCCTCCCCCAGGGCCAGCA  
GACTCCTTTGGGACTCGGGGCCCTGAGCCGCCCCCACTCGCAGGACTCACGGGTGTGCGGTCTGGGTGAG  
TGGGGGCTTGGGAGAGGTTCACTCTTGTCCGTGGGTGGGGAAGGCTGAGAGTCATGGTGTGACAGCGCCCTC  
GGCTGCCGGGTGGGGGCTCTCCCTTCTCCGAGCCAGATCCCCGGGTAC

Contig 5 (1730 bp)

CGTACCCGAGAACCCAGGCCACAGGCCTTGGCTCAGCCCTCCACCCAGGCCACGTTCCGCCCCCTCTG  
GGAAGTGGAGGACAGCCCGCCCTCGCCCTCGGACCTGGCTTCGTTGGCCCTGGCATCTGGCAGTGGCCGGCAG  
CTGCGTTACGCCCTGGATGACACCCCTGGCGTGAGCGGTGGGTCCCCGTGCTGAGGGCAGCCCCACACAGCT  
CTGCTCACTTGCCTTGTCTGCTCCGCATCCCGTCATACACATGCCATGCTGGGGCACCGTAGCGCCTTGC  
CCTGTGTGGCACTGTGGCACTGTGTTCTGTATGGGAAGACTGAGGCTGGGGTACGGCCGCTGCTGCCACCC  
TCTAAGGACATTCTGCCGTTGCAGCTGCCTCCAGG  
CTGGCCCCCGGATTGCATCTGCTTCTGGCACGATGAAGTGGCACCTCTGCCTGACCATTAGGGCTGTATTT  
GCCTTCTCCTGTGGCAGTAAATATTTACTGTCCCTCCCTGTTCTCCAGGGCCGANCAGGTTCTTGGGGG  
ATGGGAGGTGGACACAAAGGTGCCAAGCAGCCCCCTGCTCTTGGGGCCAGTGTCTGGTGGGGCCAGCCT  
GGGAAGGAGGAGCGAGACTAGGAACAGAGGCTGTGTTCTTGGGAAAAGGCCCTTGGCAGAGTTCCGGCTGG  
TGTGTGTCCAGCTAGGCTGTGAGTCTTCAACTGGGGAGCCCCGGCCCTGGACCCAGGCAGGGCTGCACCCCT  
GGTGCCAGTGTCTACTGGGTGGGCACCTGTCCCC  
ACCAGGCAAGGTGGTCCGAGCGGTCAATCACAGACAGAACAGCAGAGGGCGCCAAAGCCCCACTTTTGACAA  
ACTCCCCCTTCCGCTGAGCCGAAAGTCCAGGCGGCAGGTGGACCTCTCTGCAGGGCTTGCCACCCCTGCTGC  
CGCTTGCCAGCACTACAGGGGCTGCGGGGGGTGCCAAGCCGGCTACCCCTGAGCTCTGGAGGCGATGGA  
GTTTAGGAGGGAACGAGGGGACTCCTGGGGGTGACTTTCTTACGCGCCACATTGCGGGCCAGCAAACCGAGG  
CTGGAGGAGGCCGGGCACCTGTGCCAGCTGGAGCCTTTGCTGAGGGTCTCAAGGCTGGGGAATTGAGGC  
TGGGGGCTGGGGGTGTCACTGTGCGGCCAGGAGG  
CCCCTCGCTCTGATTGGAGCCGCTCGGCCACTTGAGCCAGGAGGCTCACATGAGGCGGGGGCTGCAGGGACA  
GGACCTCGGGGCCCGGGAGGCTTGGAGGGGTCCAGCTGGGCCAGGGTTCGTTCTTCCGGGTCCATGTC  
CACCGCCCTCCCGCTGCTGGAGGAGAGGAGGTCCAGGGCAGAAAGAAATCCGTGGGGATGGGGGGTGGTCAG  
GGGTCTGGGAGCTGTGGAACAACAACAGACAGCGAGGTCTGGGGCGCCCGGCCCGCCCTCCGGCA  
CTGTTGTTTCTGGCCGGGGTGCAGGGACAGCGAGGCAGATTCTTCAAAGTGGAGACTGGCGGGGGGCCCT  
CGGCTCCTCAGCTACCCCTGAGCTAGCCCGCC  
ACTCGGCTCCAACTCCCGCAGGCCCTGGCACGGTCTCCAGGAGTCCACTGAGGGGTCCCCAAAGCTGCCAC  
CAGGAGCTGGGCTGGGTCTGTACACACCCACCCACCTCCAAGTCTGAGATATG

Contig 6 (4833 bp)

ATGTGAGCTGCACAGCATGAGCCCTCGGCCCACTGCTGTGGCCTTGGCGACATTGAGGTGTGTGCCGCCAG  
GGCGACCACACCTGGCCTCTCAGGCTGCCGTACAGAGGCGGCTGGGTCTGANGAGGTGCGGGGCTCTGGGG  
ACCGCTGGTGTGAGTTAGGACGGGGTCAAGCCCTCCTCTCTGAAGGTTTGGTGGGTGGCCCTTCTCTTAT  
CGTGATGACAATACTGATTTCTGGAAGAGCCAGGTGTTTCTGAGGCTGTGGTGTCACTTCTCCAGTGCCCA  
CAAGGTGCCGGGCTCGGGTCAATTGAGAAGCCCTGCGGGAGCGGGTGTATGCGCCAGATTCACTTGCCT

FIGURE 6, CONTD.

CCTGCGGGTCTGGGGTCAGGACGTGGTCCCCAGCAGTCTGCTCCAGAGCCTGTCAGTGATGTGTGGGATTTTAC  
CCGCTAGAACACAGTTTCTCTGATTCTCAGAAACCAGCAGATGCTTTAGGAGGGGCGTGCAGGTTTACCTG  
TGCTGCANNGCCCCCTGCCACCTGGTCGGAGCCNCAAGACGGCATCTAAAGATCAGTTCTCATCATCAGTTC  
CGCAGTGTGGGGTGGGGGCAGATGAGAACCTCAGGGCTGGGCGCAGAGGTGGGGAGCCCGCTGGACCCCGA  
CACTGCAGGGGGGCTCCCCCTTTAGGAAGAACAATGTGCTTTGCCACCCAGCCCTCTCCCCAGGGTGGCC  
CGAACTGTTGCTCCTAAGACCTCTGGGCTGTGTGCTGTAATTTCTATAAGTGGCCACCAGGTGTACAGAGGAG  
CCACTTAAGCATCCATGTGGCGGAAACCTGGAGCTGGGGGTTCCTAAGGGTCCCTCGAGTGTCTCTGAATAA  
ATAGGCGCTGACCTGATCCCCAGGAAGGGATAACCTCTCCCAGGCCCTAAGAGGCAGTGGGGCAATGAGGTTT  
ATGTGTCCACTGTACCCCCAAATTTGCTCTTCTTCCCTCTACCCCTGTGTCCCCACCCTGGACGATACACGGA  
TGCGAGGCTGCGGGTCACAGCCCTCACAGCCCCAAAGCTGCAGGTCCCTGCCTCAGGGGCACCGCAGCTTGGC  
TGGTCCCCCTTGGGTCTCCCCACCCTGACCCGTCTCTGCTCCCCCTCCCTTTGCTTAAATGCTCTGCGTTTC  
AAGGTTCTGATGGAATAAAATAGCCCTGCACCTGGTGTGTTCTCTTTGGGGCTGTGCCAGAAGTGGGAATTC  
GACCAGGGGCAGAGCTCAGATTCCACATACTGTGTAGGGATGGCAGGTGCCACATTTCCAGGAGTTTCATTGG  
TGGTTTGTAAATGCTACTTCCGTTTCAGCCCCCTCAGCTGCCACCTCCTCAATTTAGGGACCCCCCTTTGG  
CGGTTTGGCCATGGAACCACATCATCTGGCGTGGGGTGAGCCCTTTATCCTCCCTGGCCCCACTGGGAGGGTT  
TGGGGAAGTCCCAGCTAAATTTCTCCGTAGGGACCTGGAAGGAGCCCTTGTGACATCTGGGCACAGATAAGAG  
GTAGGGGGGCACAGGCCGTGAACACTTGAAGCTGCAGAGCCAGAGCAGAGCCAGGAGCAAGTGACTCTTC  
CCCACCCCAAGAACTGTGGGCTGCGTCACACACTCCCCTGTGTGCCCTGGACCTGACAGGGCCCTTTAGCCT  
CCCTGCATCCCTCCCCACCCAAAGAACCCAGTGAGGCACCCACTTGGCCCTCCTTAGTGTGTTATGGCTCTG  
GGGCATCTGCATTTTGTTTAGGACACCCCCAGCTAGATTTAAGTCCCCCAAGTGTGACTCTTTCTCCACTG  
AAAACCCCTGTCCCTCCCAACAAAGGGCCCTATCCCTTTAGCTGAGCCAAGGAAATTCAGGAGGGGCTTGAATG  
ACAAAGGAAGAGGGGGAGAGTTAAACCCCAACACTGGCTGGCAAGCTGGGTGGGGTGACACCCAGGGTGCA  
GGGGTGCAGTGAAGGTAGCGGCTGGTGGCTTCTGGAACTACATGTGACTTTGCCATTAGGTGAGTCTTTGC  
TTTGCCCCCTGCTCTATCTGCAGGCTTATGGAAGAAGTTTAAATTTCCAGGGACACTTGGTCTAACAGGCAGC  
GCTTGTATCTGGGCCCTTCCCCAGCTGCTGACCACTCTGAGTCTGCGCCTTAGTTGGAGTTTGGCCAAGCTC  
AAGAGGCTGTGGACCCAGTCATCCCACCCAGGGGTGCCTGTGGGCAGGACGCTGCTGCCCTGCCATTTGCTGC  
AGTATTGTCACTGTCCGGCACACACACATGGTGAGGGGGTGATCAGGTGCCACTGGGGAAGGGAGAAAA  
CTCCCAGGTGAGTCCCCGCTCTGGAAGCAAGATGGACATGACCGCACTGTGTTGACAGTGCATTGGGAGGC  
CCCGAAGAAAGATTTTTCTGATCTTTCTCGAACCTGCTTTTCCCCATCATGCCCCCCCCCATTTTACCCGT  
GCCACGCCCACTGGTGTGCCGGGGTGTCAAGTGAAGTGAACAAGTGTCAATCTACTGAGGCCCTGCCACTCTCC  
ACCCCCACATAGTCCCACCTCCCAGCTGGCAGGGGAGAACTTCCAGCTAATGCCCATGCCACAAATGCTTT  
TCTGTACGCTAGAGCTGGACCAATCTCCACCTGTAACATGCTGTGCCCTGGCGTGGGAAGGTGCCAGAGC  
CAGTTGCCCCAGCAGCCCCAGAACCCTAAGTTGGCACAAAGCTACCCAAATTTGGAGGGGCTTGGGGAAGGG  
CATGGAGGGGATGAGGAGGTGAGGGGCAAACTAATTTAGTTAGCATTTGAGCAGGTGCCACGCTCAGCGTG  
GAGAGGCTCTCTTGCTTCTAGGGACCCATTATGATGCACACGCTAAAAGCGCCCTTACCATTCTCTCCAGCCT  
CAGCTTTGTCCCCCTCCTCCTCCTCAGCGGCAACCCGGCTGGAGGGTCTGGCCACTACAGCCAGAGCGCCCC  
TACTTTGGTGGCAGTCTACTATTGGCCCAACACGCGGATCACCGGCCAGGCAGTTTTCGGCAGAGAGTCTGG  
GGCACCAGTGAATCCCCCTCCTCTTTATCCACACCCAGGAGCTTTCAGGGACTACACAGCGACTAGAGGGCA  
GGTAACTGGTCTGCCCTCCCTAGGGCTGCCCTCAGAGTGTGTGAGAAAAGCTGCATTGAGTGTGAGTGTG  
AGGTGGGCTGGGGGCTTGGGGCAGCCAAACAGGAACGGCGGGACCTCTGCTTCCAGAGGACCCAGATCTGGC  
AAGCTTCGACTTTGGAGGGGACAGGAAAGACAGGTGGAGAGGGGACACTTCCCTCTTCTGTACAGACGCCAC  
CCGGAGCCACAGAGGCTTTTGAAGGAAATAGGTTTCCCTCACTAATGCAGCAGGCAAAATGGGAGGGGCA  
GGGTGGAGGGTAGTGCCCCCGCCCCAGCAGGAGGGGACAGCTGTTTCTGCAATGTAAAAAAGCAGGGTTT  
TTCTGTGTGAGAAGTTCCCTCTTGCTGCATGTCCCCACCCCGCCACCAAGACAAACAGGACACTGTGCAGA  
GGGGCCAGAGCCCCGAGATTTTGGAGTGTGTTTTATATGCATATATACCATTTTGAAAGCAAAGCTTCCCTCT  
CCCTACTCCCTACATGTCCCCCTTACCAAAAAATCCACACGTAAGTGGAAAGGGGAGTGAGAAGGACGA  
CGAAGGGGCACTGTCCCTCCCGTCCACAGCGGGACTTAAACGTACAGCTTTTCCGCTCCGGACAGTGTGC  
CGCCCCCTGGCCCCCTCAGCTCCCTGCCCGGGGGCTGAGTGTGGGGCCAGGGCTGTCTCCAGGCATGC  
ATTATTTTGTGCATGAAGGTTTTGTCCCGCCACCCAGGCTGGTGTGGGGGAAGGGGTTTATTGCTCCAAA  
GAAGCCCATCTCCCCCTCAGCCACCTTTCAGCCGCTTCGCAAGGCAGAGCTGTGCTCTCTGCTGTGTGCTG  
GCCCCCTCCTTGCTTCTATTCAAGGTGGAAGTGTGGGGGGAGGAGAAGAGTTTTATATTGTGTCTGTGATC  
CCCCGAGGCAGGGCATTTGTGTGCGGCCCCCAGCCCCCAGGCCAGGCAGATGGGGCAGCCTGCCCGACAGA  
AGGGTCTCCTGCTGCTTGGCTGCAGGAAACCCAGCTCTGGGTGAACCGTGGGCACCTTCTTCTCCATGCC  
CTGATTTTAAAGAGGAGAGCTGGGGGGCCAGAGGCAAGGAGGGGAGCCACGGCCCCAGGTCTGACAAGT  
GACCTGCGGGCTCTCCACCAAGAGTCGGGGTGGGGGGCGGATTTGGTTGAAAAGAGAACAATAGAAC  
ACACTCTTTATTTTCCCCAGGGGCGAAGAGTCAACCTTGAACCTTGAAGGACGAGCAGCCGGATTCCAGCCCC  
AGCCCCAGGGCCCCACATCTCTCGGGCTCAGCCGCGCGCCCCAGCTGCCCCCAGCCTGAGCTGCAGCAGGC  
CAGGGCTGCCCGAGACCCAGCCCCAGGTGAGCTGTGTCAGCCTGTGGCCAGGAGATCTCCGCGGCTCAG  
AAGTGAAGGCGGGCAGCCCCACCCAGCCACAGCGGTGAGTGTCTCCAGACCCAGGGCAGGGGCCGGTGTCCCC  
CGGCACAGAGAGTGTGCTGCAGGCCAGACCTCCAGGCCGTTTTAGTTCCTATCTCCCCCTGGGGGAGGGG  
TGGGGCTCAGAGGGGCTGGGGTGCATCCGCAGAGCTGGGGTGCAGGGTCCAGGTGCCTCTCTCCAGGCCGG  
TGGCCCGGAGGGGGG

Contig 7 (2014 bp)



FIGURE 6, CONTD.

CTGGTTTCGCACTCCTCCGGGGACTGTTGAAGTACCCGAGAGCGCNCGCGGAGCGCCGGGGCGAGCGGGGGTG  
GCCGCCGGGGGTGCTCCCGGGCCCCCGGACCGAGCCAGGGACGAGCCTGCCCGCGGCGGCAGCCGGGGCCGCGG  
CTTCGCCTAGGCTCACAGCGCGGGAGCGCGTGGGGCGCGGCCGCTGCCGGGAGTCCGCCTGCCTCCTCGGAGG  
CGGCCGACCGGGGAGCCTGGGGGACCCCGAGCGCCCGGGGAGCAGCGCCCCGACACGCCCGGGCCGCTCTCG  
GCTTCCTCCCTTCCAGCCGGCGCCCGCGCGGCCGGCTTCGGCACCGGGGCGCTCTCAGTGGCAGGAGAAGCG  
TGCGCTCCCGCGGGGTGGGGGACCCGAGGAAACC  
CGCACCGCCTGGAGCCGCCCGCGCGGCCAGCGCTCGCGTCCCCCGGGGAGGGCGCCACTGCTCCGCGCGCG  
CGTCCCCCGACGCCCGCGCGCTTCCCGGGCGGGCCCGGGATCCTAACCTCTCTCTCGTCCGAGCCCCGCAT  
CCCCAGGGCTCCAGGCCCGCGGCGACTTGCCCGCTCCTCCCAATTGCAGACACGACTTTTTCTGGGACCTCCC  
AAAGGACAGCCTGGCTCCAGGGTCCCCCAGATACATTACCATTTCTCCAGATCACAACTGGGTTTTTTCGGGC  
ACTAACTTCCAGAGACCTCAAAGCACATGAGCCCCCTACTGGCTTTCCAGGTTTCCACTAGTGGCTCGGTCC  
CCACCTCACTGGGGATTGTCTCCAGGCTCTTCGC  
GGTGTGATCCACCCATTTCGCGCCAGGTCCCGCAGTGCCAATCCCTCCTCTAGAAAACCTTAAACACTGACTC  
CTGGTCTCGGGGTGAGGCTGCCCAATGTGCTGACTCCCCAGAAAGTATACAGTGTCTTTCTGGCATTTGGG  
CACC GTTCCCCCAAACAGTGAAGCTCTTTCCCGCGTCCCCATAATTTTGACGCCAGGGGACCCCAAGCT  
TAGCGCCCTGTTTGGCTCCCCACACCGGAAGCCCTGCTCCCTGGGGTTCACGACAGTTTGGGACTTTATC  
TGCCAAGTTCACAAACTGATTGGCCCAAGCTGGGGTCCCTAAATTGTACACAAAGAACCCAGCCCCCCCC  
CCCAACTCCAGTACAGGAAGCGATGGCCCCAGGGA  
CCCTCGGAGTTGGAACGTGGCTTCCTAAGCCTTCACCAAAATTGAGGCTTTCCGCGCATGGCGCGCTGATGCC  
CTTGCTGAATCAGAAGCACTCTGCCCTCTGATTCTGCTTTCCACAACCTTGAGAGCATGATTTCTGGTCCCC  
CAAACCTCACTGAGCAAAATCTTTTGTGGGGCTGCAAAGATAGGAGGCATTTCTCTCCGAGCTCTCCAAA  
CTCCCTTGCTAGATCAAGTTCCTTAAACTTAGACAGAGCTTCCAGGCCCCAGAGGCACACAGAGCCATT  
ATTGGAGCTGCGTTTAATGATGACAGGGACCATGGGTCTGACAGCTCCCCAAGTCACAAATGCCCGAGGTAT  
CCTTGGCTCCAGCCAAGCCAAAGCAAACTCTTGC  
ACAGATCCCATATCTTGTTATGTCAAGCGCTTTGCGTGTCCAGTAAACAAATAGTCTGAGTGTCTTCTCCAC  
CTCATAACATTCGGAATATTAATAAAATTCCTTGGGCCCCCGAGCTGACAGACAAGAAATCCGGGCTTCTTAA  
ATTCAGAACTGATTCCCAAAATCCCGAGCCCAACGCCAGACCTCTCCCAATCTGGAGCCCTCCGACTGGACAC  
ACTGGACTCCTAAGTATTACGCGCTGTCTCCAGGCACCCCAATGCATTCAAAGTGACGCTTTGGTACAGA  
AAGGCACTGATTTCTTGGGTCCAAAGCAGCCCATGCACCCCGAGTACCCCAACTTAGTCAGCATTTCCC  
GGGTCTCCCTCCGCACTGCAAACTCCCAACTGCGG  
ACACCGGTCTTCAGGACCCACCGCTAGACGGTCTTAATCCCTTTTCCCCAGACCTAGATTCT

Contig 8 (371 bp)

AGATTCAAAAACCTATTTTCTGGGGCTCCAAATTGAGGTGCTGCCTGCCAGTCTCCAAAATAAACTGAGGG  
GTTTTTGTGTTGTTGTTTTTTTGTGTTGTTGTTTTTTTTTACCTTCCACGAAACAATCCAACTTTTTTGGA  
CCATTGATTTATGGGTCCCTGACTTTATGACCCTTGCCCAAGTCCCCCTAAATGTAGGCCATTTTCCACGG  
GCCTCCCAAAATGAAATGCCCAGATCCCGCGAAAAAATATCCCGGGTCTGGAATCCAGGTATTACA  
GGCTCGGGCTGACACCCCTCCTTGCTACTAACCAGGTTCCTGAAGTTTAGAGATCACTACCTAATGAACAA  
ATCCAC

Contig 9 (2415 bp)

CCAAAACCTGGGGCCCTATCTTACTAGGGTTCCTAAATGCAGACAGCGCCCGGAAAAATAGGGGCGTTTTTTT  
TCCTGTTTGCCAAAATAAACTAATTGAAACCAATTTTTAGAAATAAAATCTAAAATGACCTTGATTTCTGC  
GTTCTCCAAATGTACTTTTACAGCCAGGTTGCCCCAGTTTAGACGGTGTGCTTGAATCTCTAAAGCACC  
CTGAGGATTTTCCCGAGGAAGCCACCACTACGGAATTTACTGTCTTCCGGGCCACAAGCCTCCAGGCC  
ACCAACTTGGATTTCTAAACCGTGGAAATCAGCCTCCACTTCCCTCCGCGACCCCGAGGGTCTGCTCAGACCC  
CCCAACGTGCCGCTGTCTTCTCCCCCAAAAT  
TTATTTAGAGAATATGCCTCTCTCGGGTCTGCCAAGTTTCCGCTGAGACTTCTCGGTCTATCCCCAAATCC  
TCTTCCCGACAGTCCGGGAGCCCCACAAGCTTACCGACCCACATGCTGGGGTCCCCCAACTTAAACGCGATC  
CCCTGTCCCCCAGATTACCGAGTGATTTCCCTGGTCTCAGACTGGGACTCTTTTACTGGAGTCTCGAATTT  
AGCCATTAAATCACAGTTCTCCACTCCGACGAGGCTCCCTTGGGTCCCCACGTCCGGGACATGGGTTCTCTTG  
CCTGCAAAATCAGGCTGCTGACTTGCAATTCAGGCCTTTGGGCATTGTTCCCCGCGCGCGGTCTCGGTTC  
TCCCCCATCCCGCGCACGAGGGCACTGGGTCTG  
GGCCTCTTGGTGTCTCTACAAGTCCCGGAGCTCCTCGGACTTGGGAACGTCTCTTGCCTTCCCCAAATAC  
ACTCGGCCCGGCACTGTGTCGCCAGGACGTAGGACGAGCTTCTCCCGCTCCAGGAAAACGACTGGGCATTG  
CCCCAGTTTCCCCCAAATTTGGGCATTGTCCCTGGGTCTTCCAACGGACTGGGCTTGGCCCGGACACTGC  
GGACTGCCCGCGGGTCTGCTCACTTCAGCGCGTCCACCGCCGCTGCAGAGCGCTCGCTCTCCGTCTCTC  
GGCTCCAGCGCGCTTGGGACGACGCTCCGGGCTCCAGCCTTGCGGTGAGTCCCCGTGCGCTCGCGTGT  
CCCGGCCCGGCTCCCAAAACCACTCGCCGCGCTC  
CGCTGGGGCTGGCACTGGCCTCCGGCGACTGCCGGGACACGGGAGCGGAGCGGGAGCCTGCTGCAGGCCA  
GCCGCTCGGCGGGCGCGCCCTGAAACGCGCGCGGCTTTGTTGCTCTTTGCAAGGTACAAACCGTGG  
GGAAAACGCTCGGCGGCCCGCCCAAGCGGGGACGAGGCGTTGGGAAGGAGGACACGCGGAGAGGAGCAGC  
CCCGCTGGGGCGGCGCAGCGCGCGCTCCAGCCGCGGGGAGGATCCCGGAGGCGCGCGGAGCGCGG  
GCGAAGTGATTGATGGCGGAGCGAGGGGCCAGCGGATCGCGGGCTTCCGCGGCGGCGGCCCTTCCCTCG  
GAGGACTCGGGCGGCCCGGGTTTCTGGGGCGGG



FIGURE 6, CONTD.

CCACACACACACATGCATTACACACACACACACTCGTGCATACACACGTGCGCGCGCACACACACACACA  
CACACTCTCTCTCTCTGTGGGATCCCTGAG

Contig 19 (500 bp)

TGGCTCTGGCATAGGCTGGCAGCTGCAGCTCTGACTGGACCCCTTGCCCTG  
GGAACCTCCATATGCCGTGGAAGCGGCCCTAGAAAAGGCGAAAAA  
AAAAAAAAAACCAACAAACAAACAAAGCCAAACACACAGAAGCTC  
ACAGACACAAGAAGAGACTGGTGGTTGCCAAAGGTGGGGTCGAGGGTGGG  
AAAAATGAGGAGAGGGGGCAAAACACACAAACGTGCAGCCATAAAATGGT  
AAAGTCCCGGGGACCTCCGGTAGCGCGTGTGGGGACTCGGGTTGAGAACA  
CACCGTGATGTGTATTCCGCGAGTTGCTAAGAGTCCCTGTTGGAGAAACAA  
ATGCGTATCGACGTGTGGAATGAAAGTTAACCCGACCTGCTGCTGAT  
CACTTTGCAACACATACAGACATAGAATCATTATGTTTTACCCCTGGAGC  
TGACAGCGTTATACGTCCCCCAGCCTCAATTTAAAAACAGCGTTGCCGTG

Contig 20 (400 bp)

TTCATACTGTGCAATGCCAGCCTTAAATGCACAGAGGAGAGCATTAACTT  
CTTTGCAGAATCACTGAAATGATACTCATGTTTTGCAACTTGCACTT  
GGGGCTTATTTTATTGGTGCCGGAACAGCGCGGATGTGGCACCAAACTAG  
CGCCGCTGTTTTTATTTCCCTCGGTATCCGCGCTCTCGCTGTCTTCCCC  
CCCTTCCGCTTGCAGCTGAGGAAAGGGCTGAGAGGAGGAAAGTCTGCATT  
CACCCTCTCCCCCTGCCCTCTGTTGTCATCCTTACAGAAAGTGGTGGCCT  
GTGCGGGGAAGTCACTAAACCTAGGCAGGTGTCCCGTGGGGTCATGCTTG  
TTACACCTTTGTGCACCTGGCCCAAGTTCTGGGTGGAGCGAGAACGTGGC

Contig 21 (559 bp)

AGCTAGCCCCCAGCCAGGGCCAGGCCTCTCCTGCCACCCGCCAGCCA  
GCATGTCTCAAGAGGAGGGGGCCTCTAAGGGATGAGGACCTGCTCCAGTC  
GGAGACACGAAGCCCCCGCGGCTCCTCCCGAAAGTCCAGCTGCGGCTTT  
CGAGCACGGCTGCGCCCTTCGTCAATCATTTACGCCACAGAAGTGAAAGG  
CGCTTTCTGTTGGCCGAGGCAGGCGGGACAGAAATGGAATCCACCCAGAG  
GCGAAGAGCCGCCGTGGGTGAAGCGCTCTCTGGTGGGGACCGGGCCGGG  
AACTTCACATGGGGTGCCTGTCCCCATCTCCCCATCGTCATTACTGCAG  
GGGCTCGGCCACACCCGGAGCTGCGGGGGCCAGTGTGACACTGGACCT  
GGCTCCGTCCTATGATGTATGGGGGCGGGGCCAGCACAGGGCAGTGGC  
CACACCTCGGGCCTCCAGCACCAGCCAGGATGGCAGAGGGCCCCACCC  
ACCACGGGGCATGTACATCCAGAGGACCAGCTGAGCAAGGCTTGATANG  
GGCTTCAAC

Contig 22 (450 bp)

CGTGACGGGACCCGTGCGGGCCTTCCTGTGGCCACAGAGAACAACACAC  
CATTATCTTCAGCCCCACCGCGCGGCTGTTAATGGGTAAACTGGGGCAA  
GGGGGCCCCCTGCCGTAGGGCCGGGGTGGGGAGCGCAAGGCATGGCCTGTGT  
GCCCCAGCCCAGTCCCTTCAGGGCGCTGCTGTCTGCACCGGGGGCCCCAG  
GAAGCAGAGCACCAGCTTCTCCCTATTCTAGAACCAGCCCCAGAAC  
CTGGACCCAGAGCCAGGCCAGGGGATACTGACAGAGCCAGGCAAGGCG  
GCCACTCCACACCCACAGAGGGGCCAGCAACCCAGTCACTGCGCAGC  
CCATGCCCAGGGGGCAGATGGGACACGAGAGCAGCCCTCATCCACAGCAG  
GCAGGGGAGTGAAGTGGTGCAAAACGGGGCGGTTCCACGAAAGTTAAGCA

Contig 23 (535 bp)

TGCCAGAGACCTCAGAGCTGGGCTCTGCCTTCCCGGGCTGACACGGAGGG  
CTGTGGCTTCCACCACCCAGGCCACAGCCAGCCTGCCAAGTCCCTGAA  
GTGTCCCCAGAGGTGGCCCTGCCTCCACGCCCAACATCAGGCCTGCTGCA  
GCCCTGGACGGCCCCCTGTCCCCCGAAGCCCTCGGGGCTCTCTCGCGTC  
GCCTCTGGGGAACCCCTCGGTAATGTGGCCCAGCCGTGCAAGTGGCCGGATC  
ATTGCTCAGGGGGGCCCCAAGGCAGGGGGGTGACACATCCGCAAGTACCG  
CATATGCACAGGATATGGATTGGGTGTGGATTAACTTTTCGCAAATGT  
CTCTGCCGGTACAAATATTGTTTCTAATCCTCTGCCTCCCTGAGCCGGTG  
AGTCTGCCCGGAGCTGCGGGGAGCTGGCTTGCTGAACCTGCCCTGGCCC  
CCACCCCCAAGGGAGCCCCCGGCCAGTGTGAGGGCAGGAAGCTTGGGCA  
CAGGCTGCAGAGGCCAGCGCTGGCCTCAGTCACCT

Contig 24 (868 bp)

TATTGAAGACCTATCATGAGTTCACAGAGCGGAGGGGTGGAAGCAGGGG  
CCTACAGCCCACTCCCCATCACTCCAGACCCGTCCGGGGCTGGTGTCCCC  
TGCCCCCTACTCTGTCTCTGGTGGGCGGACGCTCGAAGGAGGCACTCTG  
GCCTGGAGCCTGGAGGGTCCCTGAACTCCCGCTGCCACCTGGGCCCTCGG  
GCTCCTCCTGCGCTGGGACCCGCGGTGGTGGGAAGCAGCCCTGCTCAGTG  
GGAGGAGGCAGGGCTGTGGCCGCCCGCACGGCCCTGGGGGGGACGCACG

FIGURE 6, CONTD.

CAGGACGCANGTGGGCGTGTGTGAGTCCGTCTACACGTCCAGCCAAGGGC  
GGCCGCGACCGGCCAGGGTGGGCAGCCCCAGCCTCAGCAGGGCGCTCTCT  
GGGGCTCAGGCTGCGCCGACGGGAGATGAGGGGTGAGGCGCAGTCTGGGG  
CTGCTGCCGAGAACCTCGCCAGCTGGCAGCTGGGCACAGGGAGACCTG  
TACTCCAGAACCTGAGGCTGGACGTCCGAGACCCGCGTGCCGGCCTCTT  
GGGTGCTTGGTCAGGGTCTCTTTCTGGTTGTGGGCAGAACCTCCTCAG  
CGCGTCCCTGTCATGGGGTGCTAATCACGGAGTAAGGAGCCAGAGAATGAG  
GCACGGAGTATCCAGTGTAAACCCTGGAGTATGGAGACGGGAGTACTAAT  
TGTGGAGCATGGCTCTAAGGAATGGAGTATTCGTACGGAGAACGCGGGG  
CCGGGTGAAATACGGAGAGCGGCGTACGGACAACGGGGACGGGGTATCCG  
AAGGGGAGGATGGAGTATCGGCCGAGGGTGGAGAATGGACACTAGAGGA  
TGTATANNNGGCGTCAAT

Contig 25 (500 bp)

ACCAGTTTCGATGAGCAATCCCAGCGGCGTAACATTATGGTGCAGCCTG  
GTCAATGCCGGTGGAGTTTGAACCTCCACGCGTGGCGATTGTGGTAGATA  
AATCGACATGGACCAGGGAGTTGATTGAACATAACGGTAAATTTGGCATC  
GTTATCCCGGGCGTTGCAGCACTAACTGGACGTGGGCGTGGGAAGTGT  
GTCGGGGCGTGATGAAGATAAAATTTAATGTCTATGGCATTCCGGTTGTGA  
GAGGCCCGGTATTTGGTTTGCCTCTGGTCGAGGAAAAATGTCTGGCGTGG  
ATGGAGTGTGATTGTACCTGCGACTTCTGCGCAAGAAGAATACGACAC  
GCTGTTTGGCGAAGTAGTATCAGCAGCGGCAGACGCACGGGTATTTGTCTG  
AAGGCCGCTGGCAGTTTGATGATGATAAGCTCAATACGTTGCATCATTTA  
GGTGCTGGGACGTTTGTACCAGCGGCAAGCGTGTACGGCGGGTTAAGC

Contig 26 (900 bp)

ATGTTTGATGTCCGCGCTGCTGTAAAAATTTACGCTGCTCGGTTCTTT  
GGCTTCGTCCACCACCGGAAAACGGACAAAAATTTCCGTCATACCTTTTT  
CTTTAGGCGGAAGCCAATGTCGTAATCTTCAGTAAGACTCTGCACGTCTG  
AAAGCAATACCGTACCGTACAGTACAGTGGCGTACGGCGCGGGCGGCT  
GAAACAGGTGCCGACGCTGCGCTGGGCACTTGTCCGGCGAGGGCTTCAC  
GCACCGGAACATCTTTGCCATGCAGCTCTGAAACTCATCAATGTAAGTC  
ATGCTGGTGAAGTGGTCCATTTCGCGTTCGAACGGATACACGGGATCTG  
AATCAGATCTTTACGCTCGACCAGATAGTTGAACAGACGCAATTCATCG  
GTGAAATCACATCTTCGGCGTCATGCAGAATAAAACCAGCAAAAGCGAAA  
TTGGCGCTACGCTCAAATTTGGGTGATGGCGTCCAGCACGTTGTTTCAGACA  
GTCGGCTTTGCTGGTGGGGCCAGGACGCGCGCAGACTACCTTATGCACAT  
TCGGGAAGCGAGCGCACACTTCGTCAACATCACGCTGAGTATCGGGGTCG  
TTGGGGTAGGTGCCAACAAAGATATGATAGTTTTTCGTAGTCGAGCGTGGT  
CGCCGCCAGCTCGGCCATATTGCCGATGACGCCCGTTTCATTCCACGCCG  
GAACCATATCGCTAACGGTTTTTTCATCTGGTTTATACAGTTTCGGGTAA  
CTCTTAAGGACCTAGCCCCCTATGCTGAAATGCTGTACCTCGTGCTTG  
TATCCAGTATACGACATCTATAAAAAATCGTCCAGCCCGCTGATGAACA  
TGATGACCGCTAACGTTATCGCGATTACTTTTAAGCCGTATAGCCAGGTA

Contig 27 (500 bp)

AGCTGGATGCCCCAGCTGTGGTCCCTTCCCTTCCCTCAGGGCAGGTTCT  
TGCCCTCTTGCAGCCACCGTCACGTGTGGACAGGTCTGCACACCCGCC  
GTCCACCAAGAGCGTGGCAGGTCCCTGGGCACGGGCCGCTCCTGACGCA  
CCATGTGTTCAAGGCAAGAGCACTGGACAGAGGGTCCAGACGTCCCCTTG  
TCCTGCTCAGGCCTGGGCGGGGGCAGCCCTGGCGGGAGAGGCCCTGGGCA  
TCAGAGCCTCTGTGGCCTGGAGCTTGGCGCCCTGCCCTCCCCACCTCCGT  
CCTGCTCCTCGCCGCGTGCACGGACCTCTCCCGCCCCCAGGCTCATT  
ACTCTTAAGGACCTAGCCCCCTATGCTGAAATGCTGTACCTCGTGCTTG  
TTTTCATCTGTTTATTACCTTATCTTCATTCCCTGCTTGATGATATCTGGT  
TATTCTTTATTGATTATATATATCTTGTTCGTGTTTTTATAGGACACTGT

Contig 28 (450 bp)

AGTGCGGTCCGGCCGTCCTGACGCTCAACACCGTATTTCCACGCGACCGC  
GGATTCAACCTGGTCACACGGACGCCATGTAGACATGTTTCGGGGTTACGC  
GCAGAGAAGCGACCTGCTCAACCGGCTGGTGAATCGGGCGTCTTCGCCC  
AGACCGATGGAGTCGTGGGTGTAAACCATCACCTGACGCTGTTTCATCAG  
CGCAGCCATACGTACGGCGTTACGTGCGTATTCACGAACATCAGGAAGG  
TGGAGGTGTACGGCAGGAAGCCACCGTGCAGGGAGATACCGTTAGCAATC  
GCGGTACATACCGAACTCGCGAACACCGTAGTGGATGTAGTTACCCGCAGC  
ATCTTCGTTGATTGCTTTAGAACAGACCAGGGTCAAGGTAGACGGCG  
CCGGGTACGAGAACCGCCGAGGAATTCGGCAACAGCCGGACGAACGCT

Contig 29 (450 bp)

FIGURE 6, CONTD.

TCAGGCCAATCTGTCTGGTCTCCAATGGGGACAATTTGGTTCTTTAGGCT  
TCTGTCCAATGGTCCGAATGGCCCACTCCCCGGGCGCCGCAAGGGTCC  
TCTGTGCCTCGGGTGGGCTGGCAGGACCGCCCCAGGGTCGTGCCAGCC  
CCGTCACCGGGGCCAGAAGCTTCGGGCCCTAGCTGGCTAGTCGGGCTG  
CTGTGCAGGGGGGCTGCGCTGGGGGCAGAGGCGGGGGTGAGGTAAACCTC  
CCAGCCGCGGGGTCCCTGCCGAGCCCTAGGCGCCGAGACGGTGGCTG  
GGTCGGTACCGCCAGACCCGAGGGCCTCGGGGCCGGGTGACCCAGCTG  
TCGCACACGCTCGCAGCTCTCTTGCTCATCAGGGCTCATCCCTCTGGACC  
TCTCTACTGCCCCACCTCACCCCGCCTGGACCCCATGAAGCCCCGCGGA  
Contig 30 (600 bp)

TAAAACTAGCTCTAGTAGAAACATTTTATTTAAAAATAAAAAACCTGACT  
ACGTCGGGAGTTCCCGTTGTGGCTCAGTGGTTGACGAATCCGATGAGGAA  
CCATGAGGTTGCGAGTTCGATCCCTGGCCTCGCTCCGTGGGTGAGGATC  
CGGCGTTGCCGTGCGCTGTGGTGATAGGTGACAGATGAGGCTCGGATCCTG  
CGTGGCTGTGGCTCGGGTGTAGGCCGGCGGCTACAGCTCTGATGAGACCC  
CTAGCCTGGGAACCTCCACATGCCCTGGGAGTGGCCCTAGAAAAAGGGCA  
AAAGACAAAAAACAAAAAGAAAAAGGAAAAATAAAATAAAAAAGACTATGT  
AAATGAAATTAACGACTGCCTAGGGTGGGATTTACAGCATGGGAAGTACA  
GCATGGCCGTGACAGTGCAAGGGTGAGGCGGGAAAAATGGAATAGGTTAG  
GTGAGTTTCTCCTGCTATTTGTGATGTGGTCTGCTATCGCTTGAAGACGG  
ACTGCAGTGAGATAAATATGTACAGTAAGCATCCGAAAAACCGCCAGAAC  
GGCAAAACGAATGACTCCAAGTAAGAACCCAAAAGAGAAAAGGAAATAAT  
Contig 31 (450 bp)

GCGCGGGCGTTCCGGCTGGGGTATTTAACGTGGTCACCGGTTCCGGCGGGC  
GCGGTTCGGTAACGAACTGACCAGTAACCCGCTGGTGCGAACTGTCGTT  
TACCGGTTCCGACCGAAATTGGCCGCCAGTTAATGGAACAGTGCGCGAAAG  
ACATCAAGAAAGTGTGCTGGAGCTGGGCGGTAACGCGCCGTTTATCGTC  
TTTGACGATGCCGACCTCGACAAAGCCGTGGAAGGCGCGCTGGCCTCGAA  
ATTCCGCAACGCCGGGCAAACCTGCGTCTGCGCCAACCGCCTGTATGTGC  
AGGACGGCGTGTATGACCGTTTTGCGGAAAAATTGCAGCAGGCAATGAGC  
AAACTGCACATCGGCGACGGGCTGGATAACGGCGTCACCATCGGGCCGCT  
GATCGATGAAAAATCGGTATCAAAAGTGAAGAGCATATTGCCGATGCGC  
Contig 32 (450 bp)

GGTGGATGCTGGCGATAGCGTCATCCTCGCTTATGCCGTGCAGCGGGCAA  
GGATAAAGCGCGGATAAACATGACCCGGCATCAGCCCCATGCCCGCAGA  
GTACGGATTACCTTGCCGGTCAGCGCCAGCGTGAATGCGTGCGCCGT  
GATACGCGCCGCTAAAAGCGATGGTGCCGCTACGTTTGGTGGCGGCGCGG  
GCGATTTTACC CGCTTTTCCACCGCTTCGGAACCGGTCGTAACCAGCAG  
CGTTTTCTTGGCGAAATCGCCCGCACCTTCTGATTCAATCTCGCACA  
GCTCCAGATACGGCTCGTAAGCCAGCACCTGGAAGCAGGTGTGCGACAGT  
TTTTTCAACTGCGCTTCCACCGCGGCCACCACCTTCGGATGCAAGTGCCC  
GGTATTGAGCACCGTAATCCCGCCGCGAAATCAAGATACTCACGGCCTT  
Contig 33 (500 bp)

ACGTGAGGTTTGGGGGAGGAAAGCGGGGACGAGCAGCCCCGAGAGGAGTG  
GGGGCTGGCCTGTGGCTGATGAAACTCTGAGAAGGTTAAGAGCCCCATT  
TTTGTCTTCTCTTTTTTATTATGGAATTTCAAATGGATGCAAAAGTC  
CCAAACCTAACTGGACATCTTCTTGGTACCAGGAACGGTCAGGCACCTTAT  
GATGCACCGAGCCCCGAGGAAAAACCTGCCGTCTGGAGCCACGGTC  
CAGCAGGGCACACAGGCCCCAGCCCGCAAGCGGCACGGCTGAGTCAGTGA  
ATGGCGTGCCCTCTGGTCAAGGACGGGCACTCTGGACCCAGGGAAGCCT  
CTGAGGAGCCCCCTTACAGCGTCAAAACTGTTAACAGGGCCATGTTTCG  
CACCCCCCACACAGTGGTTCAGAAGCAGACCCAGGCATCGTAATATG  
TCATCCGTGAGTTCCCTGTGTGCCACCAACAGAAAGCCCATCGTCACGTT  
Contig 34 (400 bp)

CGGCATCGATGTACATGGTACGCAAGGCACTCGTAAGGCCCGAGCCTCT  
AGGCCTTGTCAATTGTACCTGCTGCTCGCGGGGATCAGCAGCCAGGCTTG  
TGACCCCGGCCACTTTGACAGATAAGGACACAGAGAGGCCACAGCACTGG  
TGTGAGGCCCCACAGCCAGCAGCCAGGGCAGGGAGGACTGGGTCTCACC  
TGCCCTCAGCTGGGCCCAGCCTCCCTGGGAGTCCCGGAGTCTCCCCAGCTT  
AGGAGTGTCCCTGGAACCTCTTCTCTCCCTTCCCGCCCTCACCCGGAC  
CCCTGCTCCCTCCCTCCCAACCCCTCCCTCCTTCTTTCACCTTGAG  
CTCCCTCTGAGGACCTCTACTGTTCTGCTTATCCTCCCTTTGAGCCA  
Contig 35 (500 bp)

TGGCGGTGAATATGTCGTGCGTGAAGAGCATTGTGGTGGTAGCGCGT

FIGURE 6, CONTD.

TATATGCGGGAAGTTTAGGCGAACTGGACAGCCTGGGTTTATCCGGTAGC  
GAAATCCGCTTTCACGGTAAAACGCTGCTAGCGCTGGTGGAAAAAGCGCA  
GACATTGCCGGAAGATGCCTTACCGCAGCCGATGCTTAACCTGATGGACA  
TGCCGGGTATTCGTAAAGCGTTTAAAGCGATTAAGTCGCTGATTACTGAC  
GTGAGCGAAACGCATAAGATCAGCGCCGAATTGCTGGCATCGCGTCGGCA  
AATCAACCAACTGCTGAACTGGCACTGGAACTGAAACCGCAGAACAAATT  
TGCCGGAGCTGATTTCCGAGCTGGCGTGGTGAGCTGATGGCGGAAGCATT  
ACACAATTTATTGCAGGAATATCCGCAGTAAAAATCTTCCGAAGCCGGACT  
GGGCGCGCTCAGCGCCACATCCGGCTTCGGCAAACCTACAAATCCAACACC  
Contig 36 (500 bp)  
GATTTCACAAGCCTGACCCACGCGGAAATGCGCTAACAGCGTAAAGTCGT  
GCGGCCAGAATTTTTTCGCTCTTTCGCTTTCGCTCAATTCAAAAGTCAGC  
GCTACGCCATCAGCATCTTCATGATGTGATTTACGCGTCCACGGCAGGTT  
GCGGGCAAAACCGTGCGCAGGCAGACCTTGTGTGCGCGCCGACCAAAACC  
ACGGCCAGCAAACCGGTACGCCACCGCGAATAGCGACGCCATTTTGAAC  
GGTGTTGTTGTTGCTCAACCACAGAACTTCTTTCACCCGCGAGTTTCCA  
CGAGAGAAGGTGTGCGCCCTGTAATGCAAAAGAGGCTTTTACCTGGGGAT  
GATCGACCACAATGAGGTCCAGTTCATCCAGTTTACGACGGGAGAGGACA  
GGGGAGATTTGTTTCGATGACCGGAAGGGCAAAAATTTTCTTAATCATGAC  
GCAGTCTTTAACTTCATTTTATCAGGTAAAAAAGAGCGACCGAAGTC  
Contig 37 (300 bp)  
ACCTGATCAGGCTCTGCACTGTGTTTCATCAGCGGAGCCGAGATATTTGAC  
CGCCCCATGCATAACGGAAAGGCGTGGGTAAACCCCGGGCGCGTTCCCTT  
TATCAAGATGACGTTTGAATATTTCCGGCAGGTGCAGTTTGTATTCCAG  
AAAGCGGTTGAGCGCGTATGAATATAATTCTGTGGGATTTGAAGCATCCT  
TTTCCCTCCTTCGGTGAATGCGCTGAAACCGGCTTATCCAGCCGGTTCA  
GGGTACGCCCTGATAATTTGCATTTTAAATACCATTTATTGGGTACTTTTT  
Contig 38 (450 bp)  
ATCCTTTTGGGGTCTGGCAATTACGCAATAAAGAAGGCCCCCATGCGATT  
AAAGTCACCGGCCCCACTGTGCTCTAATCATGGAGAAATTTGTCCATCAGTG  
GGGTCTCGATGGGCAGGGGATTGCTCTGCGTTTCTGGTGGGATGTTAGCG  
AAAACATTGCCAGTGGTTCATTTAGTGCAAGTGTACCGGAATATTACCAG  
CCAGCGAAGCTCTGGTCCGTTTATGTTTCAAGGCTGGCGACGTCAGCGAA  
AGTGCGGATAACGGTAGAGTTTTTACGCCAGTATTTTGCCGAGCACTACC  
GGAATGTTTTCACTGTTGCATGCCTGATTTATGATTCAATTATCGGGTTGA  
TATCAGTTTTAAACCTGATTTTCTCCTTTCTAAGCCGCTACAGATTTGGT  
AGCATATTCACCTTTAATCGCGCATGATCTAAAGATAATTGAAGAGGTTA  
Contig 39 (450 bp)  
AATGTACTGGCAAAAAGCCAATGGCGAAGCGTGGGGAACGTTACATGCTC  
TGCTGGCGGATATTAATAGTCAGGGTCAGGTGCAGATGGCGATGAACGGC  
GGCATCTATGATGAAAGCTATGCGCCGCTCGGTTTGTACATCGAAAACGG  
TCAGCAGAAGGTGGCGTTAAATCTCGCTTCAGGTGAAGGGAATTTCTTTA  
TCCGTCCTGGCGCGGTGTTTTATGTCGCGGGAGATAAAGTCGGCATCGTT  
CGCTGGATGCCTTCAAAACAGTAAAGAGATTTCAGTTTTCGGGTGCAGTC  
AGGGCCAATGTTGATGGAAAACGGTGAATTAATCCGCGTATTCATCCCA  
ACGTGCGCTCAAGCAAAATTCGTAACGGTGGTTGGGATTAATAAACATGG  
GAACGCCGTGTTTTTGTGAGCCAGCAGGCAACAAATTTTATGATTTTG  
Contig 40 (400 bp)  
GACATTAATCATTTCAAATCAAAGCCCCGGTTTTTCATCGCCGTTTGG  
TGGCGTGGCACTGAACGCAATCGTTACGAGTGTAATAGTAATGCGCATG  
ATTCGTATTTCCGTTTAAATGAAGATACGGCGCGATGATACGCGTCGGG  
TTGTCTCTCTGTTGATACAGAGATACTAGATGTAGTTGAAAAAGATTCA  
ACCACACAATATATAGCCAGTAGGGGTCGAAATTACCTGGATATGAGC  
GTGACGGGTAGGGGATTTTTGTGATTCACCAGGCAAAAAGAAACCCG  
AAGACAGGCTTCGGGTCAAAGACGCGTATTTATTATCATTTTTGCACTA  
CGATTTGCGCATGCTTAACAGTGCGCCGATTAATATCTACCGCAGCTG  
Contig 41 (500 bp)  
GCAAAATCACGTCCGCGACCTGGCGTTGTGCTGGGCCATATTGGCAAAG  
GAGCTGGATTGCGGTGCCTGCAAAGTGCCCTGAATAATGCCATTGTCCTG  
TACCGGGAAGAAACCTTTTCGGAATGAACACCCACAGCAGCACGCTAAGCA  
GCAGCGTGCTGAGTGCCACGCTTAAGGTACAGCCACGGATGATTACGCACT  
TTCGCCAGTCCACGACCATAGGCGGCGATTATCCTGTGCAACATTTTTTC  
CGAGGCACGGGAGAAGCGGTTCTGTTACGCAACGACTCTGGCTGAGCA  
TCCGCGCGCACATCATCGGTGTGAGGTCAGCGACACCACCGCTGAGATC

FIGURE 6, CONTD.

AAAATCGCTACCGCCAGGGTAATAGCAAATTCGCGGAACAGTCGCCCCGAC  
GATATCGCCCATAAACAGCAGTGGGATCAACACCGCAATCAGTGAGAAGG  
TCAGCGAGATAATGGTAAAGCCGATTCACCTGCGCCCTTGAGCGCCGCC

Contig 42 (400 bp)

AGCTATCTACGGCAAAAGGCACGGTAGTCAATTTCTGTTGTTAAATACATC  
AAGCGTTTGGCGCGAAATACCATCTGCCAGATGCCATTTCAATTCGTAG  
CGCACTGCATAACGGCTACCGGATGCAGTACGTCAAACCCGAACCTGGGGC  
CGGAAGGATTTAGCTTTTCTGCAATACACGGCGGGCACCCTGGTGTGGC  
GAAAGGCGCGATGCTGACTACCGCAATATGCTGGCGAACCTGGAACAGG  
TTAACGCGACCTATGGTCCGCTGTTGCATCCGGGCAAGAGCTGGTGGTG  
ACGGCGCTGCCGCTGTATCACATTTTGCCCTGACCATTAAGTGCCTGCT  
GTTTATCGAATGGGTGGGCGAAGCTGCTTATCACTAACC CGCGCGATA

Contig 43 (450 bp)

GATTAGCGCCAGATGCTCGCCATCGAAAAGTTGAATCAACCCAGCTGCG  
GGTAATAAGTGCCGTACGAACAAATTCAGTATCCAGGGCTATCGCCGGA  
AAGGCACGGACGGCTTCACACAAAGAAGCCAGCGCATCGTCCGTGGTAAT  
CATTTGGTAATCAAATTTGTTTTCTTTAGTGGGCGTCAAAAAAACGC  
CGGATTAACCGCGCTCTGACGACTGACTTAACGCTCAGGCTTTATTGTCC  
ACTTTGCCGCGCGCTTCGTACGTAATTTCTGTCGCAAAATTTTCCGAC  
GTTAGATTTCTGTAATCATCACGAACTCCACCAGCTTCGGTACTTTGT  
ATCCCGTGAGCTGACGGCGGCAAAAGTCACCAGTACTCTTCGGTAAGC  
GATGGATCTTTTTCACTACGAAGATTTTCACCGCTTCACCACTGGAGCC

Contig 44 (750 bp)

GAGCAGCCCCGCTGATGACAGGCATGCGCCCGCTCGGCTCTCTCTCTCT  
GGTGCCTGAGTCACAGGATGGCGCGGTGGGCGCGGTGGTGAAGCGGT  
CCTGGAGGGCTCGGGAGGGAGGATGCGCTCAAGCTGGCTCCCCGTGGGGC  
TGGCCCGGAGTAGCCTCCGTGAGGGCACCGTGTCTGCTCCAGAGCCCCG  
TCCCCGGCTGCCCTGCCCTCCCTTCCCTGCCCAAGTCCCCCGGAGCCCC  
TGGATCCCGATGGGAGGCGCCCCCTGGGGAGAGGGGACCAAGGAGGGGGCC  
AGAGCTCTGAGGCCACCAGACCTGGCCAGGACCCCTTCGTGGGAAGAAGAG  
GTGGGGCCCCAAAGGCACCTAGAGAGAGGGAGGCTCTGCTGGCTGGGGGGC  
CTTCCAGGCGGGGCTTCCAGGACGGGCCAGTGTCTGGGGGCTGGAGGGA  
GTCCCTGGCTGCTGGGGGGCGGCAGGAGCACCTGGGGCGTCTGGGAAGAG  
AGCGGGAGGAGACTGGAGCCAACTGGGGGGACAGAGGAGGGGTCCAACCC  
CAGCGGTGGTGTGGGGGTGCTGGTGGTGGAGGCCCTGAGAGGCTGTGCT  
GGGGGGCAGAGCGGGTGTGGGAGGGGAGAAGGGTCCCCAGGGCTCATG  
GGCCCTTCGAGCAGTGGCAGTTGGGGTGGGTGGCTGTCTCTAGGGCTGT  
ACCACGGTGGGTGCTGGAGAAAGAGGTCTACCCCTAGTCTTTGCTGCA

Contig 45 (300 bp)

TGGGGACCCCACTCCAGCCCCACTGAGTGACGCGCCCCCTGTGGTCCCA  
CCGCCAACCTGCCTCACACCAGAGGGGCTGTGGCCACACCTTGTCACA  
GCCTGTCCCTGAGACCACGAGCCCCGGGCTAGCCCCCTCCTCACCCCT  
GGACCGAGGAGAAGCCCCACCTGGGCTCAGCTCTTGAGCTAAACTTCC  
AGGAAGTTCTGGTGCCCTCGGGTCTTAGAGCATGGTGGGAGGGGGATG  
CTGGTGGGGCGCAAGCCCTCCCCACATTCGCACTCGACCCGGTGGNG

Contig 46 (300 bp)

CCGGCTAGAAGCCACGAGAGCCCCAGGCCCCGCGGACGTCTCTCTGCT  
AGGGATTGCGCAGCCCTGGGGCCACAGGGCTGAGCAGACCTTGGGGTTC  
CGGTGTGACTCCAGCCAGGGTCCCTACTGTGTAGGCACCAGGGCAGAGTC  
AGCCCTGGGACCATGGCCACAGCTGCTCCCGCTGAGCCGGGCCCCCGC  
CCAGGCTGGGGCCCCCTCAGTGCCTGTCCCAAGCCAGCTGCTCTCCCCAC  
CTCCACCTTCTCCATCCAGGTCTGCCCCACGGCCTTTGCTCAGGCCAG

Contig 47 (500 bp)

TTGACTGGCACTAGCACGAGCTCTGTACCCGGGGATCTGGGCTCGGGAGA  
AGGGAGACCCCCACCCGGCAGGCGGAGGGCGCTGTACACCATGACTCT  
CAGCCTTCCCCACCCGACGACAAGAGTGACCTCTCCCAAGCCCCCACT  
CACCCAGGACCGCACACCCCGTGAATCCTGCGAGTGGGGGCGGCTCAGGG  
GCCCCGAGTCCCAAAGGAGTCTGCTGGCCCTGGGGGGGAGGGGAAGCAGC  
AGGGTGGTCACGGGTCTCCCTGGTTGGCAGGACCACAAGCTCAGCCCGCT  
GCCTCCAGAGGGCAGCCGACACCAACCAAGTCCGGGGACCCACGTACC  
TCAGCTGCTGCAGGTGCCCTGCCTGTACTGGTGCCAATGGGGCCGCTGG  
GTGCTCCCATGGACAGCTCGCCACTCATCCAGCCGCTACCCCCCTTCC  
GGGTCCAGTGTCCGGCCGGCCACCCGCTGCCAGCCCTGGCCTCCTCTC

Contig 48 (500 bp)

FIGURE 6, CONTD.

GGGGTTGCCGAGGCTGCTGTGTAGGTGCGAGACGACGCTTGGATCTGGC  
GTGGCTGTGGCTGTGGCTGTGGCTGTGGCATAGGTGAGCCACTGCGACTC  
CGATTTGACCCCCAGCCCGGCACTCCCACATGGCACAGGTGCAGCAGGG  
AAAATAAATAAATGAAATAAAAAATAGGTGAAGACAGTGGATTTTCATCTCT  
TGGGGTTGCGGTAAGCTCTACACAATAGGGAGTTTACCATTTTACCTGTT  
TCAAGTGGCACTGAGTCAGCTCAGTCTGAGGGCCACAGATGCCGTC  
TGCCTGGGAGATTGTTCTCTCACCACACTGCCCTCTGTCCCCACTAAA  
TACTCACTGCCCTCCCCGTCCCAAGGGCCCCCTGCCCCACCTCTGCTTCC  
TGTCTCTGAACTTGTCTGGCCACCAGCGACCGTCTGGTGACCTCACTCTTC  
GGCCCCATTTGTCGACACCCCCACCTGGCCTCTCCCCGGCATGGGCAGAN

Contig 49 (600 bp)

GGGATATTTGGGGGCATATTTGGGGGGGAGATCCCCACAAGGCATTTGGG  
GTTTGTGGTTTGAATGCCCCGGGCCCCGATGGAGGGGCGGGGAAGAA  
TCTAAGCCTTACTTGGGGAGGGTTGGGCCCCGGGGCCCCGGGCGGAAAT  
GCCCCCAAGACAGAAGGTGTACAAAATTTCTCAAAGGGTGACCCCTAAT  
GAAACGGGTCCCGGTTGGAAGAGGTCAACAGGGTGGATTGGTGGCACCG  
CAGAATTTACGACATTTTGGCTCTCTTCCAATGGCCGGACGCTTGGGGAT  
AGGCGCCCCCGTGGACGGCGGGGTCTCGGGTGGGACGGGCGGTGAGGGGT  
CGGTGACGCTTGGCCTCTCTGACCGCTCCAGCTCCTTGGCGAGCGTGCG  
AGCGCGGCGGGCGCGCAGGAGGGCGCGCAGGCCCTGCGCAGGCGTTGG  
GCGGACTGCTTCCAGGTGTCTAGCGGAAGAACTTGCCACGGGGTATCT  
GGGGAAGTTGTCTGAGAGGGGAAGGGCCCCGTGAGGGGGGGGCTGGCCCC  
CCAGGCCCTGTCCAGAACCAACCTTTGCGGGGTCTCTGCTGCTGCC

Contig 50 (179 bp)

ATCTTCATATTCATGCAGAAGACACTCTCCTGCCTTTCTATCTTGGGGAA  
AAGGACGATGTCACTTATGCAATAAAGCCCACTTGCTGGCCGGGGCTTGA  
CATTATTCCTTCTGTCTGCTGCTGACCGTATTGAACTGAGTTAATGG  
GCAAAATTTGATGAAGGTAAACTGCCACC

Contig 51 (500 bp)

CTCGGGCTGCTTCCAGGGGCTTGGGGAGCCATAGAATGCTATGGAGCA  
AGAGAGTGCTATGGTCAGACGACTTTGGGGGAAGGTCTGGGAGAAGAGGG  
GTGACTGGCCACTGTGATAAAGAGTGGGCGCTTCTTGAGATAACACGGT  
GGGCAGCCGAGGTGGACCTGTGACAGGTGGAGAAGGCCTCTGCCGCGGGC  
AGTACGTGGCTCTGGGCTGCGGACACGAGAAAGCCACCTCCACGGCTG  
CCTCCAGGCGGCCCTTCTCTCTTACACCGCGGGCCATGCCAGGTGC  
AGGTGCCATCAGAGGTGCTCAAGAGAAGCTCTGGGCTGGGGTTGTCCCA  
GTTCCCGGAAGCCCCGTGTCCAGGGGCCACCTGAGGAAGCGTGGGCGCA  
CAGAGACTGTCCCTCGGTGCTCAGAGAGGGTCCCGTCCCCACGGCAACGA  
CGCCCAAGGCGGAGGTGGTCAGAGGTCTTGGGAGGGAGGATGGCCGCGCA

Contig 52 (900 bp)

TGTGTTGCACCTGTTGCTGCCTGTGCTGCTAGAGGATCAATACTCCTTA  
CATAATTAAGGAGAACAAATGGAATTAATAAATTTGATGGGACATATTT  
CTATTATCCCCGATTACAGACAAGCCTGGAATTAAGACATAAGTTATCG  
GATATTCTACTGTTGACTATTTGTGCCGTTATTTCTGGTGACAGAGGCTG  
GGAAGATATAGAGGATTTTGGGGAACACATCCCGATTTTGAAGCAAT  
ATGGTGATTTTGAATGTTTCTGTTTACGACACCATTTGCCAGAGTT  
GTATCCTGTATCAGTCTGCAAAATTTACGAGTGCTTTATTAAGTGGAT  
GCGTGACTGCCATTCTTCAAGATGATAAAGACGTCATTGCAATTGATGGAA  
AAACGCTCCGGCATTCTTATGATAAGAGTCGCCGACAGGGGAGCGATTCT  
GTCATTAGTGGCTTCTCAACAATGCACAGTCTGGTCATCGGACAGATCAA  
GACGGATGAGAAATCTAATGAGATTACAGCTATCCAGAACTTCTTAACA  
TGCTGGATATTAAGGAAAAATCATCACAAGTATGCGATGGGTGGCAG  
AAAGATATTGCAGAGAAGATACAAAAACAGGGAGGTGATTATTTATTCGC  
TGTAAGGAAAGACAGGGGCGGCTAAATAAAGCCTTTGAGGAAAAATTC  
CGCTGAAAGAAATTAATAATCCAGCGCATGACAGTTACGCAATGAGTGAA  
AAGAGTCACGGCAGAGAAGAAATCCGTCTTCATATTGTTTGGCATGTCCC  
TGATGAACCTATTGATTTACGTTTGAATAGAAAGGGCTGAAGAAATTA  
GCGTGGCAGTCTCCTTTCCGTCCATAATAGCAGAACAAAGAAAGAGCTC

Contig 53 (450 bp)

CCAGCCACGAGCTGGACCTCCCGGAGAGGGGCTGCCTCCTCTTTCCCGC  
CCAGACGCCCCCAGCAATCTGTGGCCAAGAGGGAGTGATACCGAAGATG  
GCCACATGGGGGCGCCAGCCACAGGGAACCCAGGAAGGCGCTGGACCG  
TCAGGAGTCAGGGCTGCTGTGACCCATGTGGCCTGGGGACTTTCCACAG  
CCTGGTGGAGATGGCCGGGCACACCGCTGCCTCGGGGAACGTGCACACG



FIGURE 6, CONTD.

GGTGGTACATGTGGCCGAGCCAGGGCACAGGGTGAGGGGAGAAGGGAG  
CATGCGGGTGCAGACTCGGAGCCCGCGGTGAGGTGCTGGGTCTCAGGA  
CACGCTCTGGGAGTGGAGGACCCCATCCACGCCCTCAGGAGTGTGTGC  
CCGCTGCTCCCCGGAACCTCACAGACACGAGGGCACACCCAGCCCC  
Contig 54 (1133 bp)

ATGGCGCTCATTAGAATTCGACCTCGGTACCTTGGGATCTTTTGACCCCT  
ACCTCAGCCATCTACAACATTTACCTCCGAATGAATGAGAGACACAAA  
AGCAAATTCATAGAAGAGAAAAAAGGTAACCTGGACTTTAAAAATGTAA  
ACTTCTGCTCTTTAAAAGGCAGTGCTAATGAAGTCAAATACAAACCACA  
GACCATAAGAAAATACTTGCAAATCTTGTCTGACAAAGACTAGTGTCA  
GAACATACGACGATCAGGGAGAGGAAAAACAGCAATCTATAAACTGGA  
CAAAGAATTGGGGGGAAAAAAACCACTTGGCCAAGAAGTTGGTAAATA  
AGGCCATGAAAACATGCTCAACATCATGAGTCATTAGAAAAATGCAAAAT  
AAAATTATAATGAGATACTACTACACAGCTATTTGAATGGATAAAAAATG  
TTTTAAAACTGATTATACCCAGGTTTGGCAAGAACATGAGAAACGAGAT  
TTTCACACACGATTGGTGGAAAAACAGAAATGGTCCACCCACTTTGGAAA  
AGAGCTGGGCACTTCCCTCAAAGTTAAACATACATCCAGGACCTCACAC  
AGGCTTTCCACCACAGGTGTTTATTCAGAGACATGAAAGCGCTCATCCA  
ACAAAGACTCGTAAATGAAGTTTATAGCACCGTTTGTGGCCCGAAGT  
AGAAAACCCAAATGACCTTTAACCAGAGAATATCTAAACAAAATATCCAT  
TCACATTAATCACCATAAGAAGGAACGGGCTATGGGGACGGGAACCGTA  
TTGAAGAGGGTCAAATACATACGACGATCAAAGAAGCTGCCCAAAGG  
ACACACACTGCAGGGTTCCATGGACTGAACTCGAGAAGGTGAAACTCG  
CCAGCAGTGACAGAGAGCAGGTCCGAGATCAACCTGATGTGGAGGAAAGT  
GAACCCTCGTGCGTTGTTGGCAGGACTATAAACTGGAGCAGCCCCCTACGG  
ACAACAGTAGCCCGGGCTCCTCTCCTCCATCTCCCTGGGGAGCCTGAGCC  
TTGAGACGCTGGGGCAAGTGACGGCATGCTGCCTCACGTGGGGCCCCGG  
TGAAAACACCTGGCAGCTGGGGAAAGAATCGTA

Contig 55 (735 bp)

TACTGCCTGTCTCTATGGACTTGACTCCTCTCGGGACTTCATGCGAGGGA  
TCTTACAGAAATTTGTCTTTTGCATCTGGCTTGTTCCTGAGCATCGTG  
TCCCCAAGGTCCATCCATGTTGCAGCCTGTGTGAGGATTTCTTCTCTTT  
CAAGGCTGAATAGTACTCCACTCTGCGGATGGACCACGTTTGTATATCC  
ATACTAGTAAATCCATACTAATAACTTGTTCCTGAAGCCCACAGCTTAT  
GCTACCTTCCGTGGGCTCCTCCTGCCCCTGTCTACGCCTTCTGCTATA  
GCCCCATCCCCCTCTCATCCAGGCCACGCCTCCTGTCCCCTGGACACTGTC  
CCAGAAGCCAACCTGCCCTCTGACTGCTGCTCTGCGTGACGGAGGACAAG  
GCAGGCTCAGGGGTCCACGGGCTGGGGCCCCAGGGCTCCCCATGGCTGGT  
GCCCCCTTCTGATTCCAGAAGTACAGTGGCAGCACCAGCTTTCCAGCTGC  
CCACCTTGTGTCGCGAGGCTGCTCGGGTGGGGGCAGGTGGGCAGTGATG  
TCACCTGTGTAACCACCCTACCGTCTGCTCATCCCTGTCCAGGAGGTAC  
GGTGACCTTGGCAAACATTTCTGAACAACACACACCTCCCTCTGCTTAGAG  
GCCGGGGGCTCCCCGGGTGACTGGGGGCACAGGCTGACCCAGCCTGTC  
TCTGTTCTCTGAAGGACATGATAAGTACTGCAACA

Contig 56 (500 bp)

AGGAAGAACAGGAAACAACGGGGTTGAGGAGAAGAAACGGGTGTCTGGCA  
GGGGCACGTGCCAACGGTCCACGGGTGCTGCCGCGCTGCGGCTGGCGC  
CAGAGGGGGCAGCTCCGCCCTCGGGCCGCGCCCTGCCGCTTGTGCTGGC  
TCGCGGCTGGGCTCTGCTTGGCTGGGTACAGCTGGGTGCAGCCGCAGGC  
TGTGGTGGGTGCCGCCGGTTCAGCCAGCCCGGCCACCCGGCCCGTCTC  
GCCGGCTTGGCCCGGGCAGCCCTCCTGCACTCGAGGAGTCCGCTGACGG  
GCTGATTGGTCCACAGCCTCAGATGCAAACAGCCCCACGTGCTGGAGC  
CAGCCAGCCCGGACACCTTGGTGGAGGCAGGAAGGCAGCAGCCTGGAGA  
GCCGCGCCGGATGATGCTGCGGGGAAACCGGGCTCCCGCCGGGGCGCCC  
TGGCTCTGGCCAGGCTTGGCTTGAATGCTGACGTGAGCGGTGGCCCTATA

Contig 57 (500 bp)

TGGCGTTGCACTGGCTCTGGCGGAGGCCGGCGGCTACAGCTCCGATTGGA  
CCCCTAGGCTGGGAACCTCCATAAGCTGTGGGTGCAGCCCTAAAAAGCAA  
AAAACCCCAACATATATATATATATATATATAATTATGTTAAATACA  
CATAAAATAGAATTTACCTTCTTAATAATTTTCAGTGCACAATTCAGTGG  
CACTAAGCATTTCATGCGGCCGTGTCACTGCTCCAGAACTTTCCATCT  
ACCCAAACGAGACTCTCCGCCCATGGAACACGCCCTTGGCCCTCCCCCG  
GCCCTGCCCGCCAGCTCCTCCTGTGCTGTGGATCCGGCTCCTCCAGG

FIGURE 6, CONTD.

GACCCCGTGCCTGGGCTCACAGAGTGTGTGCCCTCTGTGACCGATCGTC  
GTGTCCCGAGGGCCGTTCTGTGGCAGCTGCGTTATGACCGACTACCTTC  
GAATGCTCAGTGACTGCCGTGCATTGGACACGCAGTCCGCTACCCTTTTT  
Contig 58 (550 bp)

TGCTTTCTGTGCCCCCTCCAGCTTGGGACCCAGCAGGGCAAGGGGTGT  
ATAGGGCTTAAGGAGGCAGGGGGCGTCTCCTCCCGCTGGCTGCCAGAGC  
ACCCCGAGCCCCGCTGCCCTCGTCCATCTCCAGCCTGTCTTTCTGT  
GCCCTCCCTGTCCCGGGCGGGCCGACACTGGCTTCCACCTCCCCACCCA  
ACTGGCGGGCCGCTCCTTCTGTGAGGCACCCGAGGTCCCGCTGTCTG  
GGGACAGCTGGCAGGTGGGTCCCAGTGTCTTCTCAGCGTGGGCTTTGGA  
GGGGGATCTGCACATACCATCCCTTCCAGCCCCGTGGGGAGCCTGGGGA  
CCATCCGGGACCCCTGTGGGCAGGCCAGAGGACTGCCAGGAAGAGACC  
AGGGGACAGGCAGTCCCAGGCCCTCAGCTTCCAGGCCAGGGGAGCCCA  
CCCCAGGTGGCAGGTGAAGCCAGGCCCCCAACCCACAAAAGTGGCCGCA  
GGGAAGTAGGAGGACAGGAGGAGGGGAGGCCAGGCCCGGGCCGCTTG  
Contig 59 (800 bp)

TGAGGACGCGCAGGCCAGGCCTGAGTGTGCCAGCTTACACCCCTGGCAG  
CTTCGTCCCTCCTGCCCTAACCCCATCCTACCCAGCAGCAGGGGCTC  
CCCCGGTGGGGCTTGGTGAGCGTCTGACTGGGGTTTGGAGTCAGGTCTGC  
TCCAGGCTCAGCCCCATCCCCAAGGGTGGCTTGCAGCACTGCTGCCAC  
CCCTAGCGCCCCCAGACCTTCGCCCTCCAGCCTGGATGTACCCACGGA  
CCCTGAAAAGTGGGGCTGAGCAGGTGCCCTGGCTGGAGTCCCCCTGACTT  
GGGGCTGGCCAGGCTGCCCTGGAGGGGCTGTGGGGGCACAGCCTGCCCA  
GGGGCCCGCTGGGCACTGGCTCTGGAGCTGACGACAGGCAGGCCCTCTCT  
TCTTGGCGGGGCCACACCTGCCCTGGGGTTTGGGGCAAGGCGGGCAG  
CCCCATGTACAGGCGGGGGCGAACCAGGTAATTACAGCCTGGCAGCCGCT  
CCCCAGACCCCCAGCCCCGAGGGCCCCCACCAGGCTGTGCCACCAAGA  
CTTGGCATCCAGGGCCCCAAGCAGGTCAAGGGCAGCTGCTACAGATTCTT  
TTAAGTTGAGACAGAATCGACACATGACAAGTTCCCTGGTTTATAGTACTT  
CGCTGCCGGGGCCGCCAGTCAGTTTAGTGACCCAGCACACCCACACAGG  
TACAATTGCTCTTCTCAAAGAGGGCCCTGAGAGAGCGCCTGTCTTGGCT  
CAGGGGTAATGAGCCCAATGGGTATCCATGAGGTTGCGGGTCCATCCCC  
GGCCTCGCCGCTTGGTTA

Contig 60 (500 bp)

GGCTCAGGAAGCGCAGGGGAGCGTGTGGGGCGACGGGAACCATGGGGGT  
CTGTCTTCCCGCTCTCCTCAAGCCACCGCCCTGCTGCCACCTCCGAC  
TCTGCAGCCAGCATGCCGGCTAGAGCCCTGTGCACCCAGCTGGTGGCCT  
CTGGCTAAGGGCAGTGTGGCTGTGGACGCGTGTCCCTCCCCAGCAGCC  
CAAGGGTCCCCTGTCAGGCTGGTGGCTGAGGTCTGCCCTGTGTGGTCC  
TTGCAAAAACCCGCCCTCTCCTGCCCTTGAAGCGTGAGGGAGACGCGG  
GCTGGGCGGATGCCCTCGGGCACAGCCGCCCGCGGTGGCGCCCTGTGAG  
GAGGGGGCTCCGACGTGCCCTGACGGCCCTGGCCGGGCGGAGAGGGTGAG  
GCCACCTCCTGGCCACGTCCACCCAGCTGCCACGCCGCTAGCCAGTGGC  
CCGGGGCCAAGTCAGCAGAGCCAGGCTTCCGACAAGCAGAGGCTGTAGGC  
Contig 61 (700 bp)

GATGAGGAAGCCGCTGCTCGTGTGCTCGTCTTCTTGGCCTTGGCCTCGT  
GCTGCTATGCTGCTTACCGCCCCAGTGAGACTCTGTGCGGGCGGGGAGCTG  
GTGGACACCCTCCAGTTTGTCTGCGGGGACCGCGGCTTCTACTTCAGTAA  
GTAGCTCAGCGGGGCACGGGGGCGGGGCGGACACAGCAGGTGCTCCATCG  
GTGCTGCCCCGTACCTGTGCGGGTCCCTTCGGATGATGGTGTGGGGGA  
CGGGGGGCGGGGGCGGCCAAGGGAGGACCTCTCCTCCGAGGGTCTGAGA  
CTTCAGACCGGGGGCGCCCTGGCCGTGCGCATTGATTGGCACCTGCCATG  
TGCCTGGCTGGGGCTCACACCCCTGACGTTCTTGCAGCGTGAATCGAAA  
CGGGAAACCGAAGGGACGGGTGGCACGGGGTGGGGAGGCAGACCGTGAGT  
GGCAGGCGTGGCAGGGGTTCTTTCGGGCGGGGTGGCCAGGCAGGCCCCA  
CAGGATGACAGCTGTCCCCTCCTGCTCCTCCTTGAACCTGCCACAGCCA  
GGGCTGCAGGCACTGACATTACCCATGGTATTGTGGTGCCTTGACGTCT  
TGGCAGTGGGCATTGGGTTTCATGACTGTTTGGATTGAAAAGTGGGAATA  
AGATGGGGTTTGAAAAACCAATTAAGAAATAAAAGGGCGCCCTGTGGGC  
Contig 62 (300 bp)

TTTGAAAAATTTTGAAGTCAAGTGCAGAAATTCGCATCTATTCCGCATTTCAGG  
CTTCTCCTGTTTCTACCTTGCCCTAGTGCGGATCTTCTATAACCACCACAG  
TGACGTTTTCAAGGTACTTTATTGAATAATAAGAAAAAGTGCACACAAT  
CATGTAGTTAACTTTCTGTGCTCTTGGCAGTTTGAAGGGACCTCTTTT

TTTCTTTTTAGGGCTTCGCGGACGGAAGTTCCCGGGCTAGGGGTTGAGT  
CAGAGCTGCAGCTGCTGGCCTACAGCACAGCTCTTGGCGCGATGGATCC  
Contig 63 (450 bp)  
TCCTGGGCCACAGGCTGCAGCAGCTCACCTGGGGGCTGGGGTCTCGCTCT  
GCGGATGGACCCATGAAGGCCGAGCCAGGTGGGGGCCGAGACGGCAGGG  
CAAAGGGTCTGCACACACAGCGTCCCCCGACCCGGCTTCTCTGGGTCT  
TGGGGGGTTGGCGAGGCTTCTCTCAGTCTGGGTTTCTGGGGAACCTTCA  
AGAAGTGGGAAGTCTTCCAGAAAGTTGGGGTGAGGGGAGGTACCCCCAAA  
GTGCTGCTCTGTCCCCATCCCCACCCCGCTGTCCATCGGCGAGACCCC  
GGACCGCGTCTCCCTGCCGAGGTGTGGGGTCCCCCCTCTGCCGGCCAG  
GCTGGGCAGGGGTGAGCGCCCCCTGCTCTGCACTCGGGACTCAGCCTGGG  
GAAGCGGGCCCCAGGAGGTCTGGCCTGGACGCGAGTGACCTTCCACCG  
Contig 64 (500 bp)  
TGTGCATCAACCCAGTGGCCACGGGGGTGACCCTCGGCCGGTACGCC  
GCCCGCTCTCCACGGAACCGGGCCTTGGCCTGAGGCAGAAGGACCCAG  
GACTCCATCCCTGCCCGGACTCTGCCGAGGGTGGGTCTGCACAGAGA  
CCCTGTGGGGGTGAGGCCGCTCGGGGCTGGGGTTGAGATGGGATGGTCAG  
GGCGGGCCCCGCGGGCTGCAGGAGGCTGGGTGAAGGAGGGGGCCAGCT  
CAGACGCCCCAAACCTAGCTTGGGAGAGCTGCAGCCCCGCCCCGTCAAT  
CGCGACAGCCTGCCACAGAGGCATTCAAATGAGAGACAAATATTGGG  
CTTGAAGACTATACCCAGCCACGTCTCTTTGGGAGCCCAAGCTGCTCCCA  
GGCCCTCATTTGGGTATTAAATTGGTTTTCTTTAGAGATTGCAATGCTTA  
TCAATGGCCACTGGGCGGCTGGGCCTGGATGCGGTCCAGGCTTTGTATG  
Contig 65 (661 bp)  
TCCCACGACCTGCCCCCTCCAGGGCCACATCTGGCGACACCGTCGCAAGAG  
TTGGACCGGCTGGTGTGGCCACAGCCTCAGGCCTTGTCTGGCCGCCAG  
GCCGGCTCCAGGCTCCAAGGAGCTCCTGCCTGCCCTCCGGAACCCAGCA  
CCCCGGGCCGCTTCCCCACAGACCTGTTTTCCAGGTCAAGGTACAG  
CTAATTTGGGCTTAAACTGGACAAGGAGGCTTATCTGGAGCAGGCTCCC  
GGCCCTTTGGCCTCTGCCCTGGTGGGAGGCCTTCCCAGAGGCTGTGTGT  
TGGCGCTGACCGTGCAGCCTGAGCTTGAACCCGGATAAGGAGGGACCCC  
ACCTGGGCTGGAGCCAGAGACCCCTCGTTCCCCAGCTCCGCAGGGTTCTC  
ACAGTCCCGCCCTGCCCCCTGGGGACCTGGACGTCGCCAGCAGGTGAAAG  
GTCCAGATGCCCTCTGACTAGAGGCTCCTCCGCTGTACAGCATGCTCCCT  
TCCCGCACCGAGGACGAGACCTCAGCAGCCCTGCGTGGCCTGGGGTGCGG  
ACCCCAAGGCGTCTCTGAGTGTGTTCTAATGGGGAGCCGTGGGGCCTCAA  
CAGTGGGGGTGGCACTTGGAGGGGAGCCTCCCCACAGCTGCCCCAAGATG  
GGCCCTGGACT

## Contig 66 (500 bp)

TTTGTGGATGAATGAATCATGAGAAAGTGATTGGACCGCCCCGTTCGT  
CCAGCTGCTTGCCAGCTGCTTTGTAAAGATGACCTCTCACCTTCTCAGAG  
GCCTGGCCGGCCGAGGTGGCAGTCAGCTGAGATGCCATGCTTGTGTTGGC  
ACGTGGGAGGCCCTGTCCACGGCGTGGGTGCCTCTTGTGTCTAATCAGG  
GTCAGGGGGAGCAGCAGGTGCAGGGCACATGTGGGGCCGGGGCCGATGTC  
TGGGGAGGGCGGGAGGAGGGGTGTGCGGAGGCCGTTGTGGGGGTGCAGG  
GGACAGACCCAGCGAGACCCCTCCCTGGCCAGGCACCAGGACAGGTGATG  
GGGGGCCGCTCCGGGGCGTGTGACAGAAGCCTCTCAGAGGAGGCCCTCC  
CACGGTCTCTGGACCATCAAGGGACCGGGGGCGCTGGGCCTGGGGGTCAC  
ACCCAGCTGGCCGGCCAGCCCGGTGGGGTCGGAGGCCCGGGCAGTTAC  
Contig 67 (550 bp)

GGGCAGGAGGGGCCCGGGGCTGGTGCAGAGGGTGGAGGTGGTGCAGGAGG  
GTGTGAGGCAGGGCTCACTGAGCGTGCGCGGCTGGCTGTGCCCTAGAGTG  
GTTAGCAGGTGCCCCACCCCTCCAGTGTGCTCTGTTACCTGTGCCTGG  
CTCAGAGGTGTGAACTGAGACTCGGGTGTTCATGAGCTTCCAGGATG  
AGAATCAGCAGGCTTCCCAGGCAGGGCTGTGTCCGGGGCTCTGGGCTCTT  
ACCAAGGAGGGGACACCCAGGGACAGCCCTGCTTGGGGGTGTGGGGCTGG  
CCAGGCTGGGTGGTCTTCTGTGGCTGGCAGCCCTTGGCAGTCACCCCC  
TTACCCCTCAACTGCCCCCTCAGCTGAGACACGACCTCCCTGCAGAGCCCTG  
TCCACCCAGACTCACTCGCCTCCTCCAGGAAGCCTTCCAGGGCTGCCT  
CGCCCTGGTCTCAGCAGGAGACAGAGAGAGAGGGTGGGCCAGGAGCAGA  
GGCAGGCAGCCAGAGGGGAAGCCAGGGGCCCTCACTACCCCTGGGGCC  
Contig 68 (500 bp)  
TTTGCATTACAGCTCGTACCCGGGATCCTTCCCGGGGCTCTGGGGGTGGG

FIGURE 6, CONTD.

GGAATGGGGGTGAGAGGCAGCTGTCTATCTGCCTGTCTACCTGCTCTCAC  
AGGCTGGCCCTGGAGCCCTGGCCTCCTCTAGGGGCACATCAGGTTTTGG  
GGGAGGCCAGCCACCGTCCCACCTCCAAGACCACAGCTGGGAGCCTGC  
CCCCAAGCCTAGACCTAGTGGGGCTCCTGCCAGCCAGGCCCCACCTTC  
ATGCTGCCACCCACCAAGGTGGGACAGTGCAGCCAGGACATCCAGCTTCT  
GGAGCTGCCCCAGGCTCAGCACAGGCTGGTACCCTAGGGAGCAGGTCACC  
CAGGGCCGCTGGCGAGGCTGCGGGGACGGGGGTAGGGTGGGCAGCAA  
AAGAACCTCTGAGCTGGGCCGGGGCGGGTGGGTGAGGGCCGGGGCCGCG  
GGCTGTGTGCGTGGCCCTGAGCCCGTGCAGACGCAGACCCTGGGTGGGT

Contig 69 (550 bp)

TGTGCTGCTGTGGCTGTGGTGTAGGCCGCCAGCTGCAGCTCTGATTCCGA  
CTCCTAGCCTGCGAACCTCCATATGCTGCTCTAAAAAGACAAACATAAAA  
TAAATGGGTGCGCTGTAAATTTGAACACTCTGCCTCCTCCAGAGACGAG  
GCCGAAACAGGCCCTCTCTGAAGGTCCACCTGGCAGGGAGGAGGAGGCCA  
GCCCCGTGGGGGGCAGAGAGAAGCCCGATGTCCCAGACACACACGCACA  
GGGACCGTGGCCCCGGCTGCCAGCCCCGGGGGGAGGGCAAGGCCAGAG  
ACTCCAGCAGCCACAGGACCTTGGTGGCCACAGGACACAAACACAGGT  
GACGGTGGGTGAGGCTTGGCCTTTCCCCCCTGGGCACGAGCACAGGACA  
CACAAGAGCCCCAGCGTGTGACCGCCACGCCAAGGAGCCTGGATGAAGC  
TGGACACCGAGAGTCCACACTGTGTGATTAGGCTGACGTGAAGTTTAAGA  
ACAAGCGGGTGGCTCAGCGCTTGAAGGCCAGAACAAGCCGGGAGGGCAG

Contig 70 (1300 bp)

ATGTCAGGATAGTAACCTGGGGTGTGAGTGCAGTGCAGATCCTTAA  
CCACTGTGCCACAAGGGAACCTCTTGACCTAGAATCCTATACCCACTGCA  
AATATATTTCAAAAAGGTAAAGTCTGAGCAGAAAAGCAAAAATGGGAT  
AATTCATTTCTGGAAGACCTTCTTGTAAAGGAAGTTTTTTGGACGTGA  
TGAAGGTAGAACTCGGAGGCACACAAAGAAAGAAAGAAAGAGCAC  
TGGAAACGGAGCAAATAAAGGTAAAAATAAAGTTTCATCTCTTCTCATTT  
TTTAATTGCTCCAAAGATAGCTGACCTCTAAAGTAAAAATAGTGGAAA  
TGTAGCATATGCTCTAGCGTAATTTAAAGTATACTTATAGCAATGATA  
GCCCAATAAAGGAGGAATTGAGAATATACAGTTGCTGTGTTCCCATTTGT  
GGCTCAGCAGTAATGAACCTGGCTAATATCCATGAGGATGCAGGTTCAAT  
CCTTGGCCTCACTCAGTGGGTTAAAGGATCCAGGTTGTCAGTGAGATGTG  
ACGTATGTACAGACGTGGCTCGGATCTGGCATTCTGTGACTGTGGCTG  
TGGTGTAGGCCAGCATCTGCACCTCCGATTTGACCCCTAGCCTGGGAACC  
ACCATATGCTGCTGGTGTGGCCCTAACAGACACAAAATAAAATAAAATA  
AAAGAGAGAGAGAATATACCATTTGTAATTTCTCACATGACACAAAGAG  
CAATGTGATATTATTTGGTATATGGTGATTGATTCAAGATGTATATCATA  
ATATTGATTCAAGATGTATATATTTCTTTTCTAAAAAAGAGATTATACA  
ATAAGGCAAGAGTGAAAATAAAGTGAATGCTAAAGAATAGTTAATCCAA  
AAGAAGGCAGAAAATGGGGAAAAGACATATAACAGATGGAACAAAATAAAA  
AAGAGCTAATGAGATTGTAATAATTTAATCCAAACATACAGATAATCCCAT  
TAAATTTAAACACTCTCAACACATTGATTAAGAAATTTGTCAAATTGAA  
TAAACAAAGCAAGACCCCACTAGATGCAGACTATGAAAAACCCACTTCAT  
ATAAGACATGGGTAGGTTTAGAGCAGAAATGATGGGGAACCATGTCACG  
CAAACATTTGTCAAATAAAGCTGGTGTGGCTGTATTCTCATCTCAGACACA  
GCAGACTTCAGAACAAAGAACTGCAAAGGATGAAAGAGATACTGCATA  
ATGATAAAGGGATCAATTTTCCAAGTGCAGGCTCCAAACAACAGAGGTTT

Contig 71 (500 bp)

ATGACCTCATACTGAATCGAGCTCGGTATCAGGGGATCTCTCAGCTGGGG  
GGGAGGGCAATGGGGCATTGTCTGAGGATGCCCCAGGGCAGGCCCATTG  
GCTGTTTGGTGCCCATGCCCCCCCCACACCCCGGCAGTCCCCCTGCTG  
AGCCTGGGACCCCTCTGGGAGTTAGGGATTGGGGGTGGGAACAGGCTT  
TGCAGTAATTCCAGCCCCAGGGCCCTTCCCTCCCCGCCCTCAGGACCCC  
CAGCCCCGCCCCACACAGTCTCCACTGTGACAGCCTCACCCTTGGGTCA  
AGTCTGTCTCTCCGGCCCCCGCTGGGCAGTGGAGCCAGCTAGGTGAGA  
GGCAGAGGCCACTAGGGCGGTGGGCAGTGTGAGGACAGAGGGGCTGGG  
TGGCCTTGGACGAGGCCAGCGACGCTGAGACAGTGAAGAGACTGACATA  
CTTTCCAGGGAGGGTCCCTGAATGTCCACTTCTTGTGACATCGGGTGAC

Contig 72 (550 bp)

AAGTCCATTAGGGAAGGGATTTGTGCAAAACACAGAGACAGGTGCAGGGCT  
GGGCCAGCTGCTGGGCTGGGGCTCCTCAAGGCGCCCGTAAACCCCTCCC  
TGCCAGCCGCTGCCGCAAGGTCTGCTGTCCACCCCGCCGGGCTGCTG  
TGTTCGCCGCGTGTCTCTGCGAACCCGACTCCCGTTACCCCTGAGCAC

FIGURE 6, CONTD.

TGCCTGGAGGCCGGCTGCCAGGCCGGGACGGGGCCCTCAGGGCTGGGCTGG  
CTCTTGGCCTGTGTTTCATTTCTGAGCAGGTCTTCTCAGTGGGGGGGGC  
CTTGGGTGAAGCAGGCATGTGCACCACTGGGGCCCTGTCCCCAGTGGGCA  
TCCTGGGCGCTTGTCTGGCCCCAAACCCCAAGGCCGTGTGCATCATACC  
TTCACCTGAGCCCCAGCCGAACCCCGACATGTGCTGGGGGACCCTGGG  
CACAGGGGTGAGGGAGCAGTGGCCTTGGTGAAGCCAGCCTTGGCACCT  
GGGGAGGGGTGCATCTGGCATGCTCTGCTGTAACCAAGCCAGGGCAGG  
Contig 73 (950 bp)

GACGTGCAGTAGCCATGACCTCTACGGCCCCCACTGACCAGCCCGTGTCC  
TTGTCCCAGAGACCCCTAAGCAATAGGATGCAGCAGAAGTGACAGAA  
CGGCCTCCGCGATGAGGTGCGAGAGGGCTCTGGCTCTGACTCAGGCCCCCT  
CATCCCTCGCTCTCTTGGAGCAGGGCCAGGTAGGGGCCCCCAGAGACGC  
CCTAGAGGAGGTGACGGGCAGCCAGCCGCCCCAGGAAGGCCTGGGGAC  
ACCAGGGAACAGAACGGCACAGGCTCCTGGCACAGTCTCCCAGGAGCCCC  
CTGGTGGCAGAGAAATCTGACCGGCCAGTGGAGGGGGCTGGGGCGGGG  
CTCGGGGAGGAGGGACTGGGTGAGGCCGTCTGACTCCTGGCTGAGCGCCG  
CATACTTGTCTGCCACGATGCCGGGCCAGGCCTTCCGCACGGACCC  
AGGCTCACATTCGCCCTACATGCCACTGTGTGGGAGTTTGGGATGGTGTG  
CCCGCTGGGGCCGGGGTTCAGGGCAGCCTTCCCAGAGGAGCGGGTTCCAG  
AAGGCCCAGGTGGAGAGGCGATAGGAGGGCTCCAGGGGGCTTCCCAGGCC  
ACCTGCGAGGACCTCCTGGGGGAAGGGAGCGGAGGGAGACAGCCGGGT  
CCCTTAGGCCAAGGCTGAGTTGTGACCGCAGGGAGAGAGAGAAGGAGCA  
CCCACAGCAGGGCAGGGGCTGCGGGAGGCTGTGCTGGGTGGCCGGGTGGT  
GGGTCTGGGGGCCAGGACCGTGGGAGGCTCGAGGGGGAGCAGGCACGG  
GAGGGGCCCCCTGGACGGCAGAGTCCCTGCTCCAGCTGCCGCCCGACCCC  
AGGTCCACCTTCATTTACAGCCTGGCCCCCGGCCGCTCTGACCGGCCCT  
GCCCATGCAGGTGTAGCGGGGAGTGAAGGCCAGGCTCCGGCCGTCCCAA  
Contig 74 (450 bp)

GCAGGCCTGGCAGCAGGGAAATGATCCAGAAAGTGCCACCTCAGCCCCCA  
GCCATCTGCCACCCACCTGGAGGCCCTCAGGGGCCGGGCGCCGGGGGGCA  
GGCGCTATAAAGCCGGCCGGGCCAGCCGCCCCAGCCCTCTGGGACCAG  
CTGCGTTCCAGGCCGCCGGCAAGCAGGTCTGTCCCCCTGGGCTCCCGTC  
AGCTGGGTCTGGGCTGCTCTGCTGGGGCCAGGGCATCTCGGCAGGAGGAC  
GTGGGCTCCTCTCTCGGAGCCCTTGGGGGGTGAAGCTGGTGGGGGCTGCA  
GGTGCCCCCTGGGCTGGCCTCAACGCCGCCCGGTCCCGCAGGTCTCACCC  
CCCGCCATGGGCCCTGTGGACGCGCCTCTGCCCCAGGCTGGGCCCTTGC  
TGGCCCCCTCTGGAGCACCCCGCCCCCGGGGCCAAAGCCTTTCATGAACA  
Contig 75 (1363 bp)

CCTCCAGCTGGGCCCGGCAGGGCACCCTGCCCCCTCAGGGGACACCACGGG  
GGGCCACAGTGGCCTCTCCTGCTCCAGGCTCTGCTCCGCTGGGGCCCC  
CTGGGCCGCCCGCCATGGCCAGGGCAAACCTCCAGTGCGGCTGCCCGTC  
TGGGCAAAGAGGCCGCCAGGCCCGCGTGGTCTTAGCAGGCACTGGCGGA  
TGCCGNTAACTAACCATTTCTTCCGCAGGAGTCCGAATCTGCTCTGACCA  
CGGGCCCTAAAAATCGCTCCTGGCCCCGAGAGGATCCCCGAACAGCGGGG  
CTGCCTCCTGCTCCTCCTGCGCGGCCGCACTCGGCAGGCAGTGCCTTC  
GTCGTCGCCAGTCTGTCAACCGTCCCGTCGTTACGATCCCCAGAGTCCCA  
CGCGCGGGCAGCTCTTTCCACACCCCGCACGGCCCCGGAGCTGCCTGGGC  
ACCCAGATCGCCCCCTGACGCCTTTGCTCCTAATTCTGCTGAAATACACAT  
AACGTCCTCTGAACGTTTGTCCATTTTACGGGGACAATTCTGTGGCCG  
TAGGTACACTCCCCCTTGGGGCGCAGCCATCGCACCATCCGCTTCCAGGAG  
GTCCCGTCGTCCCAGATGGACACTGTCCCCACTGATCCCTAATTCCCTGT  
CCCCCCCAGCCCTGCCCTTCTGTCTGTGGCCCTGGCGCCTCCAGGGA  
GCCCTGTGCGTGGGATCACAAAACGTGTGTCCCTTTGCGTCCGGTGTGT  
GTCTCTGAGCATCCGGAGCTTGGGGTGCTTCCACGCTGCGCCTGTGTGAG  
GACGTCTCTCCCTTTTGGCGGTGCGCGATGCTCCCCGTGGGGCTGCCCA  
CACTGCGCGTGTTCGCTCATCCATCCACTAAGGCTGAGTTACTTTTGGCG  
GTTGTGAATACTGCTGTGTGAACACGGGCGTGCAATAACCTGCTGGAGGC  
CATGCTCTTAGGCCTCTCGGGGGGCACACCCAGAGCGGATATGCTCAATA  
AGGTAATTCTGTGTTTAGCTTTTGGGGAACCATCAGGCTGGTCTCCAGA  
GTGACGGAGCATGCGTCGCATTACAGGAATGGTCTCGAGGCTTTGAGG  
TCTCCACCACTCGCTTCCATTTTCTGTGCGTCACAGCCGTGGAACGGC  
TGGGTGGTGCCTCTGTGTGGCTTCAATGTGCTTTTCTTTTCTGGCTAT  
GAGTTGAGCGTTTTTTTATGTACTTGTGCTGGCCATTGCGAGGGTTTTTGGG  
GTTTCTTTTCTTTTTTGGCCTTGGGGACGGCGCCAGAGCGTATAGAACT

FIGURE 6, CONTD.

TCCCTGGCTGGGGACTGAATCAGAGCTGCAGCTGCCAGCCTAGCCCACAG  
CCGCAGCAACGCA

Contig 76 (500 bp)

TCATGCCATCGCCACCGCCCCACCCGACGTTTCAAACACCAGAACCA  
CCCCTCGGGCGGCAGAGAGAGGACCGGAAGGAGAGACAGCCTGGTCCCAA  
GGCCTCGCCCCGTCTGTGTCTCCGAGCGACATTTCTTTCTGTTTCCCTC  
CTCCGCGGTCCAAGTTTACCCATCAGAGGCGCATTGTTTTATCATCTG  
AAAAAAAATCTCTGTCTCTTAATAAAACACAAGAAAAAGTAGCCTTCGA  
AAGAAAGCACATGAATGATATGTCTGGCGACAGTCTGGCGGCCTCTGA  
GCCGTGGTGGGAGGTGGGAGCCAGCGGAGCCCTGACCGATCACGTGACC  
CACGTCTCTCCTGCACAGCTGGCTGCACCTGCACGCGGTGACACAGGGAC  
CCAGCCTCCTGCCAGCAGGTACCCCCACCCCGTCCGTCTCTGTGGAAGG  
GGCAGCGTTGCCTTCTGAGGGTGGGCTGCTCTGAGGGGCGTCTTTGGCC

Contig 77 (626 bp)

GCCATGGGCTGCGGCGGTTACGCGGCTTGCCGGCCTGCCTGGAAGTCCC  
ACAGGACCAAGGGGAGGGCACGTGAGCACAGGGGCCCGGGCACGGACGG  
TGCCGCCAGCCGCCCCGCCCCGCCCCCTCCAGACAGGACGCCCCGTACC  
TTGCGGGGACAGCCAGCCTCGTGGCCTCGAGCAGAAGAAGTGAGAGTGGG  
GTGCACAGGGGCCCCCGGGGAAGGAGAGGGGACAGCGGGGTGAGCGGG  
TGCGGGCGTGCTCGGGACAGCCCTGGCCTCTTGCGCCTCCCTCCCCG  
TCCTTAAACCGGGCCAGCCTCTGGGCCTCGACCCAAGGCTGTTTGGA  
AATAGGTGGACCGTGGCCCTGACCCGAAGGCCAGCGGGGACCCGAGTGGC  
GTCCCCAATGGATCAGCAGGCGCTGGGCAGCCTGCGGCCCGGGACCCG  
GAGACACAGGTGGGAATGGGAGGAGGAGGAGGAGACGGGAGGAGAGGAG  
TGAGGACCAGCAGAAACCACGCCCTCTCTCTTCCCGTCTCGCCCTCGC  
CTCCGACAGCTCCGACTCGGCTGCAAGGAAAAGCCCCAGCCAGCCCGC  
CGCCACCGGGGGGGGGGGGGGGGGGGGG

Contig 78 (500 bp)

TACTCGGTTTGTGTACCACTGAGCCACAAAGGGAGCTCCTAAAAATAATA  
ATTTTCTTAAAGCCAATGACATGGAGAGCAGTTAGGGTGGAGGCTGGTGG  
GTGGTGGGGCGCGGCAGGCGCCCTGAAGGTCTGAGTGGCACCTTGGC  
CGGGGGAGGTGGGTGGGCGAGGGGTGTTGAGAAGGGGACGGGCCCTGCTGG  
GGGCAGGAAGGAAGAGCCAGTGGCTCCCAGTCCCCTGACCTTGCTGCCTT  
GAGCCTGGTTCTCCCCAAAATTCTGTCTGTGTCCCTTCACTTACGGAAG  
CTTGGGGCCCGTTGCCAGGGAGACAGATGGGCTGGTGACACCCAAAATGA  
GCCACCAGGAGGGGGGCACTGACTTTAGCCAGCCGGTCAATCAAGAAGC  
AAACAGGCCCCCGCTGCTGTAAAGGCAGCTTGGGGCTGGGGTCCGGGAG  
CACCCCTGGGCTGGGGAAGGGGGTCTCTCAGGCCCCCGGGGAGGATG

Contig 79 (427 bp)

TCTATTCCCGTGGCCGGAAGAGGCTAACCCTACATTGACCGGGCATCTG  
GCGATGTATCACTTCTCTCCAACCGAACTTCCCGGCAAACTTGCTGCG  
TGAAAACGTTGCGGATAGCCGAATCTTCATTACCGGTAATACAGTCATTG  
ATGCACTGTTATGGGTGCGTGACCAGGTGATGAGCAGCGACAAGCTGCGT  
TCAGAACTGGCGGCAAAATTACCCGTTTATCGACCCCGATAAAAAGATGAT  
TCTGGTGACCGGTACAGGCGTGAGAGTTTCGGTCTGGGCTTTGAAGAAA  
TCTGCCACGCGTGGCAGACATCGCCACCACGACAGGACATCCAGATT  
GTCTATCCGGTGCATCTCAACCCGAACGTCAGAGAACCGGTCAATCGCAT  
TCTGGGGCATGTGAAAATGTCATTCT

Contig 80 (650 bp)

GGCGTTGCCGTGAGCTGTGGTGGGCTCACAGATGGGGCTCAGATCCCGC  
GTGGCTGTGGCTCTGGCCTAGGCCGGTGGCTGCAGCTCCGATTGACCCC  
TGGCCTGGGAGCCTCCATATGCTGCGGGAGCAGCCCTAAAAAAAAAAAAA  
AAAAAAGGAAGAAAGAGAAGAAAGAAAGAAAGACAAAAGTCAAAG  
GAGCTCCCTGAGCGATGTCTGTCTACGAGCAGGTCCCTGGGAGCCTGAG  
GCAGGGTGAGCCTGGACCCCTGAGGGCCACTCCAGACTCAGTGCTCTCAC  
TGGCCAAAGGTCTTTGGGGACCGGCTGGGGGCGCGCGCAGGCTAAGGAGGA  
GGTCAGAGGAGGGGCTTCAGGCTGCAGGGCCAGCGGACGCTCTGGGCCCG  
GGGCGGGGGGAGATGGCCTGAGGGCCTTGCAGGGGCTGGAGGGTGGGGG  
GCTTCTGAGAGTGGGAAGACGGGAAGCCAGGTGAGAGGAGAGGAGCGAGG  
GCTGAAGCTCCTGGAAGGCGCTGGCTACCCCCAGCTGGCCCCCCCCGCTG  
CCACATTCAACAGCCACCCGGCCTGTGGTCTTGGCAGGGTCTTGGCAGAA  
AAGCCCCAAGGGGCCCGAGCCTGGCCCTTGGGCCCTAAGAGCCAAGCCCC

Contig 81 (550 bp)

TTAACCACGGAGCAAGGCTGGGGATCGAACCTGTAACCTCGTGGCTCCT

FIGURE 6, CONTD.

CGTTCGGATTCTGTTAACCACCTGCGCCACGACGGGGACCCCCAGGGCTGGC  
GTTTCCCTCTGTGTGCACACAGTGGACCTGAGCCAACCAGCAGGGCCTTC  
ACCACCACGGCGCAAGAGTCCGCGAGCAAGAGAGCAGTGTCTCATGGCTCA  
CTTTCTCCCCCTTCCCCGGAGTGGTGACAAAACCCCGCCGCCACCGGACT  
CGGTTAGACAAGGCGGTGCCAGTGGCCCCGTCTGTACCCGACGCGCAC  
GGCGCTCTCCTTTCTTCTCGGGGCTCCACCACGTGTCTCAGTTTCCGC  
ATGAGAGTACCGCGGTGGCGGGGTGGTGGCTCTGGGGTGGGGGGCCGTG  
AGGGCAGGGCTGGGCTGGGGGAGGCAGGTCTTGCCCCATTACGCGGGGGG  
CAGACTCCACATCACACGCTCTCTGTGCCTCTTGCTGCTGACACCATG  
GACTTCAAACAGGAACAGCCGTGGAGGCATTGCAGCCCAGGGCCCCGGTT

Contig 82 (550 bp)

TGACACCTCCAGGCAGGAGGTGCAGGCTGGGGTCCCAGGTAATGGTGTG  
CTGGCCTGTGGGGCGTGGGCTCAGCTCTTAGGATGGTGGGCTGGGCGCCG  
ACCCAGCAAGGACAGGGTGATGGCAGGTCGTGGGCTCAGCAAATGAGTGC  
CCAGGTTGTGGGGGTGGGCACTTGGGGCTCAGGGGAAGCTCATCAGCTTG  
GAGAGGGACGGGGGAGGGAGGGGGCCTTGGCCAGCTGGCCAGATGCCTG  
GATGTGAGCACTCACGTGCCCCGGGGTCCACCTCCCCTCCAGTGCCATCT  
GGGCAGGAGGCTCCGATGCCTGTCCCTGGGACCGCTGTCTGAAATGAG  
GTTCACTTGGTGCCTTCCCCAGAGATGCTCGTCCGGAAGCTGACGAGGC  
AGGAGTGCACAAGGGTCTGGGGAATGGAGCAGAGTGGGCTGGGGCACA  
GAGGCTGCCCCAGCCTGGGAAGATGGGGAGCTTTCAGGGGTACCCCGC  
CAGCTTGTGGGGCCCTGGATACCCAAGGTGTGAAGAGGCTGAAGAGCGA

Contig 83 (984 bp)

CTGAGCCAGCTATGTAGATTAGACCCCGTCCGTCCCAAATTTCTCTCA  
AAGCTGTCCCGAGATGAGAGATGAGGTTTTCTGTCTCTCTCTCTCTCG  
CTTCCCTGGGATGTGCCCTAGGGTGGGAGAGGGTGTGTCCAGGGCTCA  
GCAGGCGGTCCCATCTTCCCAGACGGGAGAGATCCCTCCTTCTCGGCG  
CCTGTCCCCACGGCCCCACAGACACCCCCCCCCCGGCATGGCACCCAT  
GCACCTGCCATCGTGCCCACTAGGGGATGGGTTTGGCGAGACTGGAGATG  
GCTGTAGCCAGTGAGACATGCCCTGCCACGTAGCCTGACCCCTGGGTGT  
GCTCTGTGAGATCTGGGGACCCCCAGCACACCTAGGGATCATCTTGCCA  
GCCTCCTGGGAGCCTCTCAGAAATGGGGGCCCCAGAAAGGCTGGCAAAG  
GTGATGGGAGCGTGGGAAGTCTGGCGGTTGGCGGGGTGGGTGGGGGCA  
GTGCGGGTGGGTGGGGGTGCTCCGGGTCGGAAGTGTCCAGCAAGGT  
TTTGGACACAAAGTCAGGAGGAAGGAGTGACGAGGAGACTTGCAGAATTA  
CAGGTAGAATCAGGAACCCACATCGACGCCAATTGATCTATCCCCCCTT  
TGATTGTTTTCTCTCTGGGGCTTTTTTCNTTTTTTTTTTTTTTTTTT  
TTAATCCCTCCTTAGCTTTTACGCGCTCAACACCAAATTAACGTAATC  
CCCACCCACGTAACAGGGGGGCGGTGACCCGAAGGACGAGGAGCACACG  
AAGCCACCATCCGTACCTTGGCGGCACACCGCTGTCTGCCCTCCGC  
CCATTTATCGCCCTTGAATTGATTTTTGTTTTGCTCTGTCCCTGTGCTT  
GGGTAGAGTGGAAAAGGGAACCTCTGTGGGGGTGCCAGCCACTGGGCCCC  
CCAAAGATTTAGGGGAATGAAACGGCTGCCGCC

Contig 84 (550 bp)

TGCCCCAGCAACCCCTGTTAGCCACACTCGCGACTAATAAGGCGA  
GAGGTACGCGGCAGCCCCACGGGAGAAAGTGCTCCGTGCCCCCCACC  
CCTGGCTCTGATGGCCAGCCTGGCACCCCAAGGTGGCCTCGGCCTTCCT  
ACCTCCAAGGTCCAGGCGCATGTCCAAGACCAGCAGAAGCTTCTCCAGG  
GTTGGTGCCTGCTCAGGGCAGAAAGCAGGGGTGAGGCTCCCCAAAGGGCC  
ACTGGCACCAATGCCCCAGGCAGCCCCAGCGAAGGGGACAGCCACCCC  
CAGCCCGGGGACGCAAGGCTGAGGGGACATGGGGAACCCAGAGCAGGGCC  
AAGGGGAGCAGAGCCCCCTCTCGGGACTTGAAATCTTTCCGGGGGGCC  
CAGGGAGCTGGGGTCTGCAGAGGGCACTTTCAAATACGGCCCCACCCCA  
AATTGCCACGTGGGCCACAGGAGCAAGGAGTCGCTGCCAAAGTGGCCTGGC  
TTCAGCGCAGGAAGTCCCCCTCTGGGGCTCCCTCCTATAGGCACAGG

Contig 85 (500 bp)

TGAGCCAGGGCCTGGCCAGCTAAGCCCTGGAGCCCTCCCGCCTGTTT  
CCTGCCCTCCATGCTGGCGGAGCTCGGCTTACTGAGCGGGGGCCAGGCCA  
GTGTGCGTGTGGAGGTAGATTCCACTCAGCTGGAGGTTGAGGTGGGCAGG  
GGGCCGAGACCCTCAGGCCAGCTCTGGCCGGCCAGGTCCCTGAAGCTCC  
CCCGGCTGGCCTCCCGTCCCTGCTTGGCCTTGTCTGGCCCTTGCTT  
GACAAAGCTCTGTGGCTCTGCCTGCAGGAGAGACACTGGCTCCCCGCTC  
TCGGATGAGGACGGGGCTTTTCTGCACAAGTCTGCCCCAGAATGTTTGG  
GGCGCCAGCAGCTGAGCCAGCACGTCTCCCTGCCCCCTGGCTGGACAC

FIGURE 6, CONTD.

GAATCCCGGCATCGAGGCGGGAAGGGGGATGGAGGGATGGGGCCTACCCA  
CCCCTGCTCCCCACCCAGAATAGCTGGGCGGCCCCATGGGAGGCGCCCC  
Contig 86 (913 bp)  
CTGTTTTACGTCTTCTGAGGACACACCCAGAAGAGGGGCTGCAGGCGCC  
CATGGTGACTCCATGTGTCTCACTGCTGAGGCCTCTGCAGACCGTCTCCCG  
CAGCAGCCGCACCCGTTTCCATGCCACCAACAGCGTGCGAGGCGCACTG  
TCCCCACGCTGTGCAACTGTTTTGAATCTGAGTTATATAAGCAACAGAC  
GCTCCTTCAAACACACTCACGTGCACACGTGCGCACAGGCGCACAGACAC  
ACACACGGAGTAATAGGCCTCCCCCCCCCTCCCTGAGCCCAGAGGGGGCCT  
GGGGCCCTGGAGCCTGTGCTTTAGGGCCTTTAGGAAAGCTGGTGCCTCC  
CAGAGGGGCGCCCCGAGCGTTGGCTTCCCAAGTCCCCACCAACCTCGA  
CAGACTCAAACGTTGGTTTCTTTCGTGCTTTTGCCCAAGGGATGGGCGG  
AGGTGGCCCTGCCTGAGGTTTCAGCCACGCGCCCCAGGCACCTTTCTCT  
CCCGGTCCCCGGCCACTTCATGGGACAGCGGGCCTTCCCCACGTTGTCC  
CCTGGGTTGTGCTGCTTTTCGTAATGAGACGGAGGCAGGTGCACCTGTCC  
TGGGGTGAATTCTCTTCTGCAGGAACGCTTCCCGGCGCCTGGTCTGT  
CTGTTCTCGGTTGTTGGAACCTCTCGTCACCAGAAAGGGTGGCTCTGAC  
GTCGCCCTTTCCCTCCGTGGCTTTTGCAGTCTGGGTCTTGTGGGGAACC  
TGCCCCAAAGAGGGGAGTGACCCCCACGAGGGAGACGTAGCTCCTGTGG  
CGACAGCACCGGGGGCCCCAGATTTCATGGGGTTACGCTCACAGTCGCA  
TGACGCTGCCTTTGGACGAGGGCAGCTCAAGGAAGCTTGTTCCTGCCA  
CGAGCCACAGGCA

Contig 87 (650 bp)

TCCACACCTGTGGAGCCGCTGCCTCGCTGATGCCCTCTGCCAGCTGATG  
GTGAGGTGCCAGACTTGGGGCTCAGTCCAAACAGGGGCCCCACAGGTGCT  
GCACCTGGGCAAGGGAGCCTGTGCGCAGGGCCTCAGGTGTCCAGGCTCG  
CTGGGACCGAAGCGCACTGGGTCTGGACTCCGGGCTTCCCCAGGGGCTG  
CTCGGGGGCCACCTGGAAATGAAGCCCCACCTGGCTCATAGGGTCCACGTG  
AGGGCCCTGAGGCCACCAAGCCACCAACAACACTCAGTTAAGGGAGGGGAG  
CTTGGGGCTGCTAAGCTCCAAGCGGGAAGCGGCCCACTCAGCACTGCCT  
CTCTGCCAGCCAGCCGCCAGCTTGCTGACGTCCCAACCAGGCCAGGGAC  
CCTGTCCACAGATGCTGGGCCCTTCCAGTCTCTGCTCCCTGGAGGCGCT  
GGGCACTGTGTGGGCACACAGCCCGCACCCGCTGTAAGGAAGGGAAAGG  
CCCCATCTCAAAAAAGCCGTGGGCAGGTGGGCCATGATGGTCTCCGAG  
GCAGGTCTCTCTGGGACCCCTTGCTCCCTCGGGCTCGCCCAGGAGCCGCC  
AGGTCTGCCCTGGATTAACCTTGCCCCGCATGTCATTTCAAACCTGGCTT

Contig 88 (700 bp)

TGGGGCCCTTTGGGGCCGGAGCGGCCAGTCTGCTGGGCCCGGAGCAGGG  
GGTCTCTGTCCGCAGGGAGGGGGCCTGGTCTCAGGGGAGGAGAGGAGGCA  
GGTCTCACCTGAAAGGATCTGCCTTCTCCTCAGGCCTCTGGGATGCCTGG  
GCAGAGAAACCAAGGAAGGAAAGGCCCAACTTGCTGGCTGGTGGGGATGGGG  
CCGGGGGTGCTCCCGGCACACCCCCCCCCAAACCCACCTTAGTGGCCAA  
AGTGGGTGTCATGATGGCCACTGACCTCACGGGGGCGCAGGAGACAACAA  
AATTCAGCCACTCTTGGGGGAAGGACACTTGTGGCCTGAGTCTTAGGGG  
CTGAGTTTCGGGGGGGACCCCCAGCTCTCCCCCAGTATGAGACACCTG  
CCCACTCCTCCAGCTGCTCCCCAAACCCAGTGTCTTGACAGGGGCATCT  
CCCCGCTGCCCTTGCAGCCGCTGTCTCTGACCATGTCCCTCCCCACCT  
CCCCCTCTGCAGGGCCAGGCCTCCAGGGAGCAGAGCCGAGGCCACCCTA  
GACTGAGCTGGGGACCGAGACCCCAAGTCGCCACCCGGTCTCTGCGTTAG  
AGAGGGGGTTCCGGGGGGCACCTGGGGCGGCACTGGGGGGCGGGAAGGA  
GAGCCCTGGGCCGTTCTGGGAAAGGTCTGGGAGGGAGGGAGGGGTTTTGC

Contig 89 (1400 bp)

GCACACCCGGAGAACAGAGGGAGGGGTCTTACCAGTCTCAGGGTTTTTT  
TGGGGATTTCTTTGAACTTGCCCTATTGGTTTCGAGGCTTCTGTTCTCTC  
CAATCCCCCTTCTGAACCCCCCAAAATGGGTTTACGCCCCACCCAG  
CCAGAGGAAACCAATTGGGGGATTGGGGGGAGGCGGGGCCAGCAAAAGCC  
TTGGGCCCCCTAGCCCCCTGGCTTTGGCCTCTGGCCTGCCAGGTAGGGGG  
AGGGACGCGGTGACCTCCGGGGGCTGGCCACGGAATCTGCCCCACCC  
CAGGGCAGACGTGCACAGGAGGGGAGAGGCTCCGAGGAATGAGGCCATCA  
AAGGGACAGGTGAGGCCACGAGCCGTGGGACCTGGAAGTGTTTAGGGCCT  
GGGGGACGAGGCTGCGGCCTGCGGGCTCCGTGGTCAGGAGGCCCTCTGCC  
CACTGAGCAGCTCCCACTGGCACACGAGCCTCTCTGGGGTCCGGCTG



FIGURE 6, CONTD.

GTCTCCGGCAGGGGTGGGCTCTGAACGTCCAGCTCCGCAGACAAATCAGA  
 TTCCCCGAGCCCTGAGAAAGCCCCCTCCCCAGCCCGTCTCCCCACCTG  
 TCGGTGGACAGAGTGACCCCTGCTGACCCCTGCCCGGGCTCCCGCAGGA  
 GATGTGAGAGAGTAAGAGGCGGTACAGGACGGCCGGGGCGGCCCGGGCGA  
 GGTGCAGGTGTGTGGGTGTGAGGCTGGGCACAGGCTGGCACAGCCTCCCT  
 GGCCCCAGTCCCTTGGGCACCTCTGGGCACCTCGGTGTGCCTGCCTCCTGA  
 AGGGATCCACCCCTCCAGCCACCTCCTCTCGGGCCAGCCCCACCCACCC  
 CCGAGCTACAGATGCCTGCGCATTGCCCCAAGTGTCTGGACCCCTGGAG  
 CCAGGCAGCCACCCGCTCAGCCTGGCCAGACCCAGCGTTGCCCTTCACG  
 CCCTCCTCCTCCCCGCCGGTCTCGCGCTCGTCTCCTCAGGTTGGAAGC  
 CCCTTCCACCTGCCATCTTGCTGCGCCAGGATACACGGCTCAACTCA  
 AGGCCTCACTCCTCGCCCTCTCAAGGCTCTGTCCAGGCCCCCTCTCTGAC  
 CTGGCACCACTGCCGCCCTCTGGCAGCCCCAGCAAAACCCCTGCCACAG  
 TCCACGACAGTCTCTTCTGGCTCTGCCCCCAGGATGCTTCTAGAAGTGG  
 GGGGGGGTCTTCCAGCCACGCAGCATCCACTGGGCCCTGGGCTCCCT  
 CCCCAGGTGCCCCCTCAGAGCTTGACGCTGGTGCAGACGGCTGTCTCCGA  
 ACCCATGCTCCCTGCGCCCTTGGACCTGGTGAGATGTTGCAGGTCATTTG  
 GCTGCACCCAAAAGAGTGGCCCCCTCAGGGTCCCCCTGCGCCCCCTCCATC

## Contig 90 (350 bp)

GTACTGTAGGGCCCTCATTCGAATAGCCTACTAGGTCACAGCTGATCCACA  
 CCTTAGGCCATCACAACTTCCAGAGGTAGTGCCGCTCCTGTCTGTTGAAC  
 AAGACGGTAGTGACTGCTGTGAGAGCTCAGATCTGGTGGGTCACTGACCG  
 AGTGTGGAACCCCTGGGGGAAGGCTGTGGGTGTCCCCGGCTGGGTGGCCA  
 TGTCATGTGCCCCCTTCTATCCCTTGGACGAGGCTGGTTCCTCGGCTCT  
 AGAGCCCCAAGCCCCAGCTGCTCTGCCAACCCCCAAGCCTGAGCCTCAT  
 CAGACCCACCAACCCCATCGCCATGGCTACGCAGGACACACCGCTCTCCAC  
 CCCCACAGCCGCCCCACCTCCCCGAGGTTCCAAAGCTTGA

## Contig 91 (1464 bp)

TCCAGGACCTGATGCAGCAGCCACGTCCCGAGGCCCCCTCCACGAGGCCC  
 CTGTGTGACCAGCGCTAGGGAAGGGGACCAGGGAGATGCTGAGAACGGGG  
 CCTTCCGAGGGGGCAGGTGGGACTGACTGTGACCCAACTCCCCACCCC  
 CCTCTCCCGCTCCAGAGGGTGCCAGCCTGGAAGCTGGCAAAGTCCAATCC  
 ACAGGTGGGCTCACGTGGGGAGGCTGGTGGCCCCACCTGGTGGGGCCCC  
 AAGCTGCCTCTGGGCGGGGTGGGGGCTGCTCCAGCAGGGTCCCATCCAG  
 CTTCTCCCTGGGGAGACTCACAGTCTGGGAGAAGGGTCTGACTGCACC  
 GCAGCGCCCCCCCCCTCCCCAGACTACCCCAAGTTCTCTCTGTCATCGG  
 TGACTGGTCTCCGCATTTGCCAGGCTGGGCATCTGCCAGAGGATACGT  
 CCAAAGGCAGGGCAAAGCGGGCCCGTCCCCCGGAGCTCCCCACAGGCGC  
 TGAGGGCTGGGTGGATCTCGGGGGGTGGAGGGGAGGACTCAGAAGGTG  
 CAGCGGGGTGGAGCGAGGCTGAGCCAAGGTGCACGCGAGGGCCAGAGAAG  
 GCCGAGGCGGGCAGGAGGAGAGCGCCAGCCTGGAGGGGGGTGGGTGCC  
 CTGGGCAGGTCTGGGGCTCAAGAAGAAGAGAGTGTGTGTCAGGGGGCTG  
 TCCAAGCTGCCCGGAGGCTGCCTGCCACCTCCAGGGAGCAAAGCAGGG  
 AGGCTGCAGCTGGCCCGGCCGCGCTCTCCAGGACCACGCTGGCCCAG  
 GCCTCAACGCTCCTCCACAGCCAGGAGACCCAGGGCACCGGTCCATT  
 TACCGCGGGCTCCGGTCCGTTTGCTGCGCCCTGGGATGGACTGTGGGG  
 GCGGGGCGTGTCTGGGGAGGAGGAGGTGTCTGAGGCTGGACACCTTGA  
 AGGCAGGTGAGAGTGACAGGTCCGTGCGCAGGAGCCTTCGGCTCTGGATT  
 CTGGCCCTGAGCGAGGGGCTGGCTGGAAGTGGGCCGGGGCTGCCGCAGG  
 AGAGTGTGAGGGAGAGGAGACGGGGTTGGCCCCGAGGTGCCGGGGTG  
 GTGCCCTGGAGTGCAGGTGAGCGGGAAGTGGGTGTGGCGTCTGGAGACG  
 GGGGTCGTGGGCTTGGGATGGTGACAAGACCCCCAGGTGGAGGCGGCC  
 GCAGAGGAGGCAGAGAAGCCAGGCCAGCCCCAGGCCGGGAGGCGCTGGG  
 AGTCAGGAGGGACAGCAGAGCCCTGGGCTCAGTGTACCGGTCTCTGGCA  
 CCTCGCCGACGGATGTCTGGCCGTGCAGTGGTGTCTCCCTCACCCCTGAG  
 CCCTGAGAACCATGCAGGATGCTGGTGTACAGCAGGAGAGGGCCAGGGC  
 CTGGGGAGGAGTCTTACTGGAAGGCCTTCTCCTTCCGTTTGCAGCAGGCG  
 GGAATGACTGGGGG

## Contig 92 (694 bp)

TGGAGCCAGGGCACGGCAGAGCGGTCCCGAGGCCGTGCGTGCTGACCCGG  
 GGGATGGGCGACCTGGGGGTGGGCTGTGAGCCAGGCATAGGGACCCCG

FIGURE 6, CONTD.

ACTTGGGCACGGCCAGGTGGGGCCGGGCAAGGGGAACAAGGACGCTGGC  
CTCCAAGGGCCCCACGTGGGCACAGAGGAAGAGCCGACCCAGGTTGTGGG  
CGCATGGAACCCCCACTCTGGGGGCCAGGAGGCCGAACGTCCAAGGGC  
TGAGGCTGGGAGGGAAGAGTCCCTTTGGGGGTGAGTCAGTGTCCCTTGTG  
GGTGCCCCCTGCCACTGGCGGCACCTCTGACCCCAACTCCTTGCGGGTG  
GACGTTGGATGGATTTCCTGCAGCCTTCTTCTGGAATAGTCTCTGCCAT  
CCTCGGGGAAGCAGTGATTGCTCTGCCCAAGTCCAGGCCCCGCCCTGCAA  
GGTGCTTCCACCCCAATGAGCCCCGGACAGTTCGAGGGCTTCTCACGC  
TACTGAGGGGTATGAACAGCTGTCCCCCTCGGAAAGTGGGGGACAGGCC  
CTGCCACTCCATCTCGGGACGCCCCGTCTAGTCAGCACTTGTCTCCCTG  
CCTTGTGCCCCCTGACCTTTTTTGGAGACCATCAAACTCAGCCTCTG  
CCCCAGGAGGTCAAGCCCCCGTCCCCAGCCCCAGACCAGCA

Contig 93 (900 bp)

CCAGCCCCATCCCCGGCTGGTCCCCCACCACACAGAGCCCCGTTTCCC  
AGGGGACAGCACAGCCTGCCCCAGGTCTTACATAAAGTACCTTCTCAG  
AGCTCCTGTGCGGGCTCAGGGGAATGAATCTGACCAGCATCCATGAGGAC  
ACAGGTTTGATCCAGGCCCCGCTCAGCAGGTTAAGGATCTGGCGTTGCC  
GTGAGCTGTGGTGGAGGTGCAAGACGTGGCTCAGATCTGGTGTGGCTGT  
GACTGAGGTGGCGGCCAGCAGCTGCAGCTCTGATTGGACCCCTAGCCTGG  
GAACCTCCATATGCCGCGGGTGCAGCCCTGAAAGGACAAAAATAAATAAA  
TAAATAAAGAAGTAAACACACCTTCTCTAGCCATAACCACTGCCTAGG  
GGCGGAGGGCCAGGAAGCGGCACCCCCGCCCCAGGCTGCCCGTGGCGCCC  
CGGGCAGGCGGGCTCAGCCTGCTTTTGTCTGTGATGTGAGCCGCCCCAGC  
CCCACATGGAGGGGCTGGGCTGCGCAGTAAGTCTTAACTGACGGGAGC  
TTCGACCAGCAATTCAACAGCGGCATGCAGCCGGGAAGGGAAGTTATTC  
GTGTGTAGCTATTAGGCGCCGAGTGAGGGTGTGCCTCGCCCTGGGCCCCA  
CCCCTGGGGGGAGGCATCACAGGGCTTTTGAACACCTGCCCATGAACACG  
GGGCAAAAGCCAGCCAAGGGGGCAGGTGCCTGAGGCTGGGAACCAACCCG  
TGTCTCTGAAATCCGGGAATGCCCACTGCAGGCATGTTCAAAGGGTCAA  
GACCGGGGCTCTGCCTGAGAAGGACTGGCGAAGGCCAACTACAAAAGCGC  
ACCCCTCTGTGCAACCCCCCAACCAATGGAACAAACTCCAGAGGGGCCA

Contig 94 (550 bp)

AGTCTGGGCTGTGTCCATGGGGTTGCCAAGGTGCCAGGCAGAGACCTTGG  
GGACAAAGGTCTGTGAGCAGAAGGACATGGCCACGTCCCCTGCTCAGCA  
GGTGCCCAAGGCTGGGGTCTGATGCCCTCGCTGGGGTGGGGCGGGTTGAG  
GGGCCAGGCCAGACACCCCTTCGTCCCTGCCGAGTTGTTGCCCTTCTG  
TTCCTGGAAGGCCCCCTGCAGGTACAGGAGGCCCTGGGGCTGACGCTG  
CACCTTCTGACACCTGTGGTCTTGGGGATGGGACAGGACAGGGAGACCCC  
GGGGCTGGACGGAGCGGGTAAGACAGAGAGTTGACTCTGTCTCGAGTCT  
GTGCAGGGCTGTCCCGGGCTTGGGCTTCGTCTGCAGGGCCTTTCGGGTCA  
GGGTGGCCTCAAGGTGACGAAGACCTGGTCTCGGGAGTCTGCAGGCGCA  
AAAGTTGGAGCCACCCCCCGGGGAGGGGGGCCAAGGACAGGAGGGCC  
CAGGGAAGTCTGGGGCTGCAAGGCCGTCCGGGCTGGGGAAGGCCAAGGT

Contig 95 (1200 bp)

GTTTGTCTCAGCAGGCAAGGGCCTCCGAGGCCTTAATAGCCCATATGA  
CAGCGCCCGCTCCTGGCATGGGGCCCCGCTGGCATGGGGCAGGGCAGGG  
CAGAGCAAGCAGCATGCAGCTTCTACCTTCTCCTGACCTCGTGGCCCTT  
TCCGAGGCCTCAGGGGGTCCCCGAGTGGGACCCAGCCCTGGCTCTCCT  
CTCCAGAGCCAGGCCCAAGGCTGGGAGTGGCCAGAGATGAGGGTGGCCG  
AGCAGGGCACTGCCTTGGCGTCCCCATCCCTGGCGCCTCAGGGCCGTACT  
GTCCAAAACCAAAAGAAAGCAGTCAGCAAAACTTCTCCAGCAAGCTGGG  
GTCAAAGGTGCTTCCGAGGCGTGATCAGGGTGGCCTTTGCTACTGTAC  
CGTGTGCCCTGGGAGAGGCACAGGGACACAGACACACCTCCGAGAACC  
TGGGGCTTCCAGGGCGTCAGGCTGCCTGGGCCATCCCGGGCCCCCTGTGGT  
CCCAGGATCTCGGGGACCGTGAGGCCTGCGTCCCACCTCTGCCTGGGA  
CAGGCCCCACAGAGCTCACAGCCAGGGGACCGGGGACAGGGCCCCGCTG  
GGCCACCTGCCTCCAGCCTCACCCAGCCTGGGCCCCAGGCCTGTGCCTGC  
GACACCTGTAGTCTCAGGACGGGCGCGGACAAAGCCGCCCGGCCCTCC  
CCCGGCTGGGAGGAGACCCGCTGGCCCTGACGTGTGGGCTGTGAGAGC  
TGAAATGTACAGCAATTAGCCCTAACGAGGCCGAGGGAGGGAGCGGCGG  
GGAGGCCCGGCGAGGGGATCCACAGCCGAGGGGCCGGAGCTGGCCACCC  
CACCGGTGATTCCAGGCACTCAGGGATAATTGGGTGTTAGAAGTCAGG  
CGGCAGCAGAGAGCGGGCCAGGCGGGCTGTGCCCCCTCCACCGCCCC  
TTACAGGTGCCCCAACACGCAGGTCTGGGGAGATGCTGAGGTGCGCAAG

FIGURE 6, CONTD.

[illegible]

GGGGACCAGGGCCAGCCCTCCAGCTCCCACGCATACCTGCTAGGAGCTT  
GCAACCTGCGAGAGACTTTGTGGACCCCTGCCGGGTGACCCCTGAAGCTG  
GCAGCTCTCTTGCTCTGACCGGGCTCTCTACACTACCCCTCTCCAGC  
GGCTCGGGCCAGACATACCCACCCCGAAGGAAGCAGCAAGCATCCA  
CCAGCTGGGCCCTTTTCCCCAGCCTGTGACCGCCCCGCGCCCCCTCAC  
ACCTGTGCGGTCCAAGACCCCTCTCTGGCTGGGCCCTGGTGCTGCCCTTG  
CCGTGCACATCTGGGGTCCATACCCCAACAGGCCCACTTTTCTGTCT  
TCCAGTGTCCCCCTCAGTGCCTGATGGGCCACACCTGGCTCTCTGT  
CTGCCCCCTTGACCGCAAAAAGACTGGGGTCAGGACCCCTGCCCCAT  
GACTGCCCTGGAAGACCTCAAGCTCTCCTCTCAATCCTGACCTTTAAG  
GCTCTTGGCACGGAGAAAGCGGCTGGGGTGGGGGAGGGTGTGGGTCCCA  
AAGCAGTGTGCATACTATCTGCTGACTGGGAGCTCATTCTCCACAGCGTG  
Contig 97 (1350 bp)

CCCGCCTTATTTTTTAAATTTCCGAAAACAAAAACCACACCTCTCCCGTCC  
CCGAAATTATTTTGGTATAGTCTTATTCAAAGAAGTCCTGCCACTGAAGC  
CCACTTGTCTCTGTCCCGGGCTGCTTTGGCCAAGGGCCCTGACGGGCCAG  
GGTGGCTCATTTCCGCATCCCCGACAGGGCCGCTTACATCCCATCGCG  
GAGCTTGGCTTCCGGCACCCGGCTGTGGCCCTCGCTGTGGCCATGGAATGC  
TTTCGAGAAGCATAGGGGCCACAACATGGGACAGCCTCGCTCTGCTCGC  
TGTGGTTCCGCTGAACCTCTCAGCTGGACATCTGGGCAGCAAGCACCCCA  
GCTTTGCTTCAGGCTCTGGTTCAGGCTGGGCCCTCCTCGGCCCTGCCCG  
CTGGTGGCCAAAGCAGGGCTGGTCCGGCTGTGCCCCCGGGTCTATAGAAGC  
TCTGACAGGGCTTCTACAGCCAGGCTGGGATTCGGCGGCTGCCCGGAC  
TGAGGCCCTCTGAGTCTGACCCCCCATCTTCCCTCCACACAGACGCC  
CCCGCCCCCGCTTCTGCTTCAGTGAGGCCCAACCCTGCCTCACTCGCTGA  
CATTTCCAGAACAGGGGGTTCCAGGAAGCCCTGAGCCTGCAGGGGACTCA  
GTGACCAAGCCGATCTGAATTTTCCCTCCTTCTGATCTCTGGAGACACGT  
CTGGCTCAGCCTGGCTCGAGTGCCTGAGCTGGGACCAGGACACGCT  
CAGATGGAGGTCTGAGCCTGGGCAAGGGCAGGGCCCAAGGCTCAGGGAGAA  
ATTGCAGGTGTGAGATCAATGACCGGAGCCTGGATGGGGCGGCCCTGGCC  
AGGGCAGCTTTCTCCCTGCAGCTCCCTGCCACTGTCCCCCCCCAACTCTGG  
GCTCCTGCTCTGGACCCAGTTGTGTTCCTCCTCCCAGCCGAGCCAC  
CCTCCCCCATTTCTGCCCCCCCCAATCCAACACCTATCGTGGGAACCACT  
GGAGCTGAAGAAGGACCCCCCAAGGGCCCCCAGCGCTGTAATCCTTG  
GGGGCCTCTGCCAGGTGCCAGTCTCGGGCAGGAGGGGCGCGGGCACA  
GCCGTGGCAGATGCGCCCCCAAGCCTGGGCTCGGAGGAGCCCCGCCCCC  
ACTGACATTTCCAGGCCGCCGCTGCAGACCCGGCTGGCCGTGATATTTA  
GACAGGGCTTATTTGCCGTGACTGGTTTTTGATGACTTTGGGGCCAGGA  
TGAGCTCAGCCGAGCCCGCTTGGCCACCTTGGTCTCAGCTTGGGTTTG  
ATAATATAACGCGTTCACTGAACCGCTGACGCCTGCGTGGGCCGAGGCC  
Contig 98 (1354 bp)

GCTTGCAGTAGTTCATCAGATTGGACGACTCATAAATGTCAAGACATCTA  
AAGATTGGTGCATCCAATCATTCCCACCAGGTTGTTTTTTGTAGATGT  
CAAGAAGCTGACCCAAAACCTACGTGGAATGCACGTCAACTGGGAGAG  
TTGAAACAATTTCTAAAAAGAAGGACGTCGTGGGAGGACTCTTCGCG  
CTCTTTGGTTTTCGCTTCACTTTATATTATTAGTACTGATTTCTCTAAA  
GCTGCAGTAGTTCAGACAGTGGGCTCTATGAAGGAGGGGCTCAGAGAT  
GGTTGGGACAGAATAGAAAGCCAGAAACGGACCCCGCAAAATGTGGTCA  
ATTGAGTTTGGGCAAGGATGTGAAAGCGGTTCACTGGAGAAGAGTCTTTT  
CAAGAAATCTCTGGTCTTGATCCACTGCTCATCCAGGCCAAGAGTGAA  
CTTAGCCCATTTCTCAGAGTGATACAAAAACTGACTCAAAAATAATTC  
ACATACCGCTCGTGTAGCGTATGAAGCCATGAAACATCAGAAGAAAATCT  
CGGTAACCTCAGGGCACTCTGGGGCTCCACCTCAGCACCACTGGCCTTG  
GGGCCAGATACTTACGTGTTCTCCTGTGCACTGTGGGACGTGCAGCCAAA  
CCCCAACAGGTGACCATCAGAAATGTCTCCAGACGTGCCAAATAACTG  
CCAGAGAGCAGAGGAGCCCTCACTGAGAACCACAGGGTGGGGCAGAGAG  
ATCTCAGACATGACACGATTAGGGGAAAACAATCTGACACACTGGCTTTG  
TTAAATTTAAACCTTTCCCTCTGTAAAGGCAATGGTAAGACATTAAGAG  
GCGAAGTGGCAGACTGGGAGAAAATATTTGCCAAATCATGTATCAGATACG

FIGURE 6, CONTD.

AAGAAGATGCAGGAAATCCTCAAAGTTCAGTCACAAGAAAACCCAATTCA  
 AAAACCAGCAGAGCAGACATACGATGGCAAATAACCACGAGAAAGTCAGC  
 ACCCGCTGTCCCTGGGGGGACGCGAGTCAAAGCCAGGAGGACACCAGGAT  
 ATGCCCACTGCCAAGGCTACGGATAACGGGAAGCAAGAGACACAGACAGA  
 AAGGATGCTTCGGTGCTGGGGAGGGTGGGGTGGGGCGGGGGTCCCCC  
 TGGAGCAGGATGTGAAGGCACCTGGGGGGGGCTCTGCACTCCTGGGGGCC  
 TTTGGCACAGGCGGAGGGCCCGGAAGGCTCTAGGGGCACGGAGAGGGGT  
 GCCAGGCTTCCTTACCCAGCCAGGCAGACCAGGCCCTGTATGAAGCCT  
 GACGTGCAGCAGCAAGAGCAACATGCTACAGACATGTGTCTGTGTGTG  
 TGTG

## Contig 99 (1000 bp)

GGTTCCTCAGGCGCACGGGCGAGAGGCTGAGGGTCCGAGGGGCTTTGGGTG  
 CTGGAAAGCCTGAGTTGAATCCCAGCTCGGTTTCTTAAAGCTGTGTCTC  
 CACGGCCAAGGAATGGGGCCTCTCTGGGAAAGGTCTGGGGTGAGGCTGGC  
 GGGACCTGCCAGCCCCGAGGGCATCTGACCAGACAGTTCTCAAGCTCA  
 CAGGGCTTCATGGCAGGATGGGGAAGGCTGTGGTGGGGAGTGGGGAGCAC  
 TCGACACCCCTTCCAGGCCTCTTGAGTCACGGTGGCCTCTGAAAAGGGGT  
 TCTCTGTGTCCAATGAGCAAGTCTTTGTCCGGGGCAGGATTACTAAGTCC  
 AAGGGTGTCTGCCCTCCGTGGGGCACAGAGCAGGGGGCCAGATCACGT  
 GGCTGTAAGTCCAGGTTGCAAAGCCTGCCACCATGTCCACTGGGTCTCT  
 CCAGTTACCTTGGGAGGTGCAGGGTGGGGTGATGGGGAAGTGGGGCAGA  
 GAGCTGGCAAAGAGTGCAGGCGAGGACTGCGGGCGCCAGACCCAGCTAA  
 CCGACCCCTCACACGGAGCTGCTTCTACTTTGCAGCCTGGACGTGGGAAAA  
 GGTTACCCACACAGCAGCGTGTGCAGGCACGCTGGTATGTCTGTGTACTTA  
 TGCATATGTTCTACGTGCATGCACGTGAGTGTGCTGTGTGCATTGTGCCT  
 GTGTGTGTGTGCATGTGTGTGTGCACTCATGTGTCTATACGTGTGTGTAG  
 TGAATGCTTGTGCATGTGTATTTGCATGTGTATGTTGTACGTGTGCAGT  
 GAATGCATGTGTGTGCAGTGGCGGCATGTGCGTGTGTGCGCATGTGTCTG  
 TTTATACCTGTGTGTAGTGAATGCATGTGCATGTGTGTGTTACATGTGC  
 ACGTGAGAATGTGCACTCGTGCATGTTTGCATGTGACTTTTCATGTACACA  
 TGCTTTTAACGTGTGCACGTGTGCACATGTGTTTCTGTGTCCCTTGCACG

## Contig 100 (1500 bp)

CGTATAAATATATTAATATAGAATAAAATAGATTGATAATATAGATAAAC  
 TAAACCCATTATCAATACCGGGTGGCCCCAGCAAAGGATACTAGCCAGTT  
 TATCAAGGTGCTAAGTCAGCACATAGAATGGCCACAAACGAAAACCTGTA  
 CTGCCTATGTCCACTCTAATGGAGTATGCCACTGACATCAGTGGTAGGTG  
 AGCTGAGTCCATCTGGGCTCCAGTTCGGGCCCCGCTTGTCCCCAACGG  
 AGGTTCCCTCCAGGGTTCCCCAAACCAACCGGGCCCCAGGTCTCCCTG  
 TCTTGACTCGTTTCTGGAGTCTTCTGGGGCTCTGCAGTCTCCCTTGTG  
 GGGCTTCTGTCCCCCTGCCCTTGGCCTTGCAGGCTCGGCCCTGCCCTGGG  
 TCCCGGGCCTGCGGGCTCACCTCCTTCTTCCCTGGAAGAGAGGGAGCC  
 AGGCTGGGCGGGCCAGGAGGGAATGCGCCTGACTCTGCTCCAGATGGAC  
 AGGTCGGGACATGCAGTGGCCTCGCCTTGGGCTGCTGAGCCAAGAGCAGG  
 ACGGGTCTTTCTGGAATGTGGGGCCAGCCAGGTTACGCGTGTGGGTGGG  
 CAGCCGCCAGCATCTGTACAGGCCGCTGCAGGCGCGGGGAATGACCTCGA  
 CTTCTGCTTGGCACCCAGCTCTGGAACAGCCCCCTGCGGAGCCTCCGCCC  
 AGAGCTGGGCGAGAGGTTCCCTGTGCCGGGGACCCAGCAGGGCCCCCTC  
 CCTGACTCTCCAACCCACCTGCCTGGGAGGAGTGGCCCCCTGGCCTCCGT  
 GGATCTCTGGGTGCGGGCTCAGCCGGCTTGACAGCCTGGGAACAGCCAAT  
 GCACATCCCCAGGCCTGGCCACACCCTCCACCGGGAGCGGGCGGATCTG  
 CATTTCGCCAGGCTCTGCGGGCAGCTCTGAGAGCCCCGGGTCTCGGAGCC  
 CAGCCGTGGCCGTTGTACGCCCTGGGGGCTGTGGACAGCGTGTCTCATT  
 GCCCCCTCCGAGGTCCGGGCCAGGTCCCTCCCACTGCTCGCCAGAGCC  
 CTCTCCCCACCAACCACACTTCTGTGTTCTGCAAGCGGGACACACACT  
 CCGGTTTCAGGACCTTGTACAGTGGCGCTTCTCTGCAGAGAAATGCCTG  
 GAGCAGATGTTTGTCCGCACGGCTGCTCCGCGAGGCCTACCGAGAGCCCC  
 TCACCTAAACGGCCGGGCTCAGCAGCCCGGGGCCCTGTCCCCACCGCCC  
 AGGTGGTGGGTTCTCTGTGCCAGTGTGGGCATCTCTGTAAGATACCTGT  
 TTATCTGCTCATGCTCTGGTCTCCCCAGAAAGGTAGAGCAGGGCCCCGCA  
 CAGCCGTCTCGGGGTGGCCACTCGCCCTTGGGGCTCAGCCTCCATGCAG  
 GGAGGGACGCTGTGACACGAGAGCCCCGTGTGAGTGTCCGGGGCGCC  
 AGCCTGCCTTAGGTACAGCCAAAGCCGGCATTAAACCACAGGCCCTCGA

FIGURE 6, CONTD.

## Contig 101 (600 bp)

TCTAGAATACCTGGCCCTCCAGGGACGTGTCTGTAGCTGCGGCTTTTCAG  
GGCAAAGTGTAATTAACATCCCCAGGCTTCCCTTCCAGTTGGCACAGGG  
CACCACATGAGGAGCAGCCTCTGGGTGCCAAAGGGCCCACTGGTGCCAG  
GCGCTGGGCTGAGTGACCCCCGCATGCTTCCCGCCCACTCACCTGTCTGG  
CCCCACCTTGACCACAGCACCTGTGGGAACACTAGGCCTGGCAGCCACA  
CGCTGCTCTCACTGGAGGCCAGTGCCAGGCAGCCTGCTTGGCTACGCTAG  
CAGATGCCCGCTCGCCTCTGCCCTGCCCTAGCCCATGCAGGAGCCAG  
GGTGGGGCACAGGAAGGACGATTGGGGCCCCAGGTACGGCACATCCAGGC  
CACAGCCGTGGCCACACGAAGGCGGCCCTGAGGGGGCGTTGGGGGGCAGA  
CCCTGCCCCCGCTGCCGCCAGCTCCAGGCATTAATCCCAGGGACC  
TGTTGCCACTGGGTGGCCGCCAGCCTGCCCTTGCCTTCCAAGGCCTCTA  
AAATGCCCTCTTTTCGTAAACTAGGACTTACCAAGCTCAGCGAGCCCTC

## Contig 102 (1867 bp)

AGTATATCGGGTGAGACTGGGGACCGGTCTGCCGGAAGCCCCACCATAA  
AGGCCACGTTGGGCCACAGTCCGGGCCACGTGAGTGTGGGCGGTCCGCG  
GGTCTGCTCTTGGAACACCAGGATCTCTAAGAGGTACCAGCCGAGGCCAA  
GTTACAGTGAGCAAGTGAGCAAATGACTGAATGAGAGCGTGAGCGAATGA  
GTGAGGGGTGAGTCCGTCCACCACGCAGCCTAGGCTCAGCCAACCGCTGT  
CCCCGCGTCTCCACTGGTGACCAGAACGAAAGAGTGGGGAAAGAGTGGT  
TGCTCTCCACAACCCAGTCCCCAACCCCTGGACGCCCCACCCCTCCAG  
GGGTGCCGGGCTGGCCTGTGGGCCAGTCTGGAGGCTCTGGCACCTTC  
CTCATCCGTTCTCCAGCACCCAGGTTCTGTGCTGAGCCCTCTGGCCCA  
CAGGCCTCGGGGACAAAGAGGGCCACCTGGAGGCTCAGGGAGCCTCACCT  
GCCTCGTGGTCTGGCGGAGGCGGTCTGGACATGTGATAGACCGGCCTG  
GGCTCAGCAGCTCCTGCTGGAAGATGTGAGGACAGCCTGGGCCACTCTC  
CCACCAGGAGAACTTATTCCTCGGTGGGTCCCCCGGGGAAGGGATGGG  
ATCCAGCGGGGACCCAGAGCGTCCAGCACACGACCTGTCCCTCCAGC  
CCCTGCCCCACACGGATGCTCACAGCTCAGCCTCGAACACGCACCTGTTG  
GACTTTGCTCTGAGGCTGTCTTCTCAGCCGACGCGGGCTCCGCTGCA  
TGGTCTGGAAGCCAGTGGGACTCGGTGGTGACAGGGAACAGGGGCTCTT  
GGAGTGGGGTGGCGGGGAGCCCGAGGGAGCTGCTTGGGCCCTTGATGG  
CTGAGTGGGTGAAGTACAGGAGGCTCCCCAGGGCTCCCTGACCCCCC  
CACCTCAAAAAATCCAGAGCATCCTTTGCTTTGGGTCTGGTGAGGCTCTC  
TGAGGTGAGACCTGCGTGGCTGGGCCAGTGGGCTGGAGCAGGAAGAAA  
GCAGGACAGCCCCCGCCCTGGCCAGACTCCCCAAACCCAGCAGGAGAC  
ACCTGAAACGGGATGGAACCATCCTGAAAAGAGCCACCTCCTCCTCTTA  
TGCATCAGCTGCCGGGCTCTGGGGGCCCGCCAGGCCCCAGATGTCCGG  
GCTGCTCCCGTCTCACATCCAGGGGTTTCTGGGCCAGGACTCTGTCCCC  
CCAAGCATGCAGAGGTTCCAGGCTGGGGTCTTCATGCTGCCGTGTGCA  
TGGTGGGGAAGGAAGGGGACAGTCTGGAGACCCCCGCCCTCCCATGCG  
TGGCGCCGGGGACAAAGCCGGTGGGGTCTCAGGTTTGGGTTAGAGCA  
AACGTTGATCTGACCTGGTTCTGAGATGCTCGGCCGATGCTGCGTTGTC  
CGCTCGCATTTTCTGTTTCTCTGGGAGGCGCTGCGTGGCTGTGGCTT  
CCGGCCAGCCCCACGAGGACGAGGCTGGCTGGCGGGTCTGGGGCC  
CCTGCCCGCACCAGAACGTCTGGCTCAGGTTTTGTCTCGTGACCCATC  
ACTAAGGGCCACCTCTGACCCGAGCCCTGTCTCCGAGGTGGGAATTGG  
GGCTGTCCCTGGCGTCATAGGACCTGGTTGGGGGCATCCAGGCTGTGT  
CATGCCCTCCCCAGAAGACTCTGGGGGCTGCGGGAGGTTTCCCCAGCT  
TCGGGCCAGCCTGGGGAGGGCGAAGGCGCTGGAGGCCTTGCTGTCCCA  
GGGAGCATGGCTTCGCTGCAGACTGGGGCCCCGCACACCCAGCCACACT  
GGCCGTCTGGAAGCACT

## Contig 103 (650 bp)

GTTGAGGATTCTCGGCAATTTCTCTGCTCACTGGCGCTCCAATCGCCTCG  
ATGGGCTTCTCTCCAGATACAGCTGCAGATCCTGGGCGGGCACACCGTT  
GAGCGTCACCTCGTAGTGAGATTGCACTCGTTGTCAATGGACATCCAGG  
CCATGCCGACGGCATGTGGATTCTGTGCATCCGTGTGCTCCTGTGCTTC  
AGCAGAATGGGTTCCGCCGAGTCCCGAGCATCGGCCACTGGACGGGGCAC  
TAGGCGGGCCAGGATCAGGCTCGTCTCATGCTCGGTGGCCACATTAACGC  
CCAGTTTCGCCGGCATAACAGGACTCGAGGACCTTGGGACCCAACTTCTCC  
ACACTACCAATGCGCTGGTTGAAGTTGAAGCTCGGCGTCAGATCCTCCAG  
CTTGGCCTTCCGCTGCCCTGCTCTCAATCAAAGTATGTTGGGCCTAT  
CCCGGGTGTTCACGTGCTCCGTTTCGATGTTGTAGGCCAGAGATCCATCG  
GTGTTCAAGTAGACCCACGCCAAACCGCTGCTTGGTTCGAGGATTCCGGC

FIGURE 6, CONTD.

ACTGTGCGGGCGCCAGCAGGGTCTGGAAGATTTTCGCAGCTGGCTCGGGTCA  
CGATGTGTCCCTGGATGCGCAGATGTGGGTACTTCTTGGACTCCACGGTC  
Contig 104 (1630 bp)

GGTGTGTCTACTGCTGTGGCTCAGACCCCTGCTGTGGCACAGGGTCCATC  
CTTAGCCCAGAACTTGCACATGCCACAGGTGCAGCCAAAAGAAAATTCT  
TACTAATAAGTTGTTTCAATTTGCCTTTACGTAGAGTGGCATCAAACAGCAA  
ATTTAAAACACCATCTATCAATACATAGACCGCGGTCAAAGGGAAAGAAC  
TTTCTATTTTCAGCACCTTTAATCATGGCTTTGCCGAATTTGGGACCAGGG  
TGCTGTGTTTTTCATCTCTCCCTGCAGGTGGTCCCGAGATGACCAGGCCGG  
TCCTGGGCGGGAGGAGCCGGACTGTGGATCCAGTTGCTTCCCAAGACAGG  
CTGACAGGAGAGCAGCAAGGGCCACCCCAACCGAAACCAAGCCAGAAC  
GAGCAGAAAGATGCCGTCTTCCAAGTGGGGGCTGGGAGCTTCCCTCCATC  
CTCCGGAGCCGTGAGGCTGCCCTGGAGCTGGCAGGAGCCACAGAGGACCC  
GGCTTTGACCGCCCCCTCTGGGACCCACAATCAGGACCCTGACTCAGATGC  
TGAGGGGCTGGACAACACCCAGGACCCTGTGCTTCCCCAGAACCGCT  
GTGTCCATCAAGGTCCAGATGGCACCCGTGTCCCACTGGAGCACGCACT  
CCGTGGGGCAGGCTTTCCCTTGGGCACCGATGCACCTTGAGGGCAGAGAC  
GGGGCCCAATAAACGTTTCCAACACAGTGGGTGAGGGACCCGACCGGCC  
GACACGGCAGCCCGGATGCAGGGACTCCGTGCTTGGCCAGCCTCCCTTG  
GGGTGGTCTGTGTCTCAGGGGTGGATAGGCCATCATGTGGGTGGCCTC  
TGGGGACATCCGTTCTCTGATTGGGTGAGTTTCAGCCACAGAGATATTCC  
CAGGACTACAAAGCTGGGTCCCTTGGGGCACCTGCTGTACAAAAAGACA  
AGGCCCTGACCCCCAGTAGCCAAGTTCCCCCAGGGGCTCCCCAGGGTCTG  
GTCATCCAGACTGTGCCAGCCGTGCTGCCCGCCCCAGTCTGCCTGACCC  
GAGTCTCTGTAAACATCCCCCGGCCCCACCCAGCTTTACCCCAAGGCCGA  
AAGCACCAGCCCCCTGCACCACAGATGAGGCCCCCATGGCTCCCCGACC  
TAACCTCTGTCTGCAGTTGGCTTTACGCTCGGGTGGGGGCAAGGCCTGC  
ATCTCAGGCTCCCCGGGAGAAGTTGCTGCCTCCACAGCAGAGCCAGGGGCC  
TGCTGACCACCTGGGCGGGTGGATCTGGTCTAGAATGCTGCTAAGGTG  
TCCTTGACAGGCAGCCCCGGGCGGCCGCCCTCCAGGAAGGAAGGGGACA  
TTGCCAGGACTCAGGAATGAAGCCATCCAGGTTTTGAATCCCCGGTCCC  
ACCACCTTCCACCTCTGACCTCAGGCACCTCGGCTTTCAGAGCTGCCCTT  
TCTGACTCTGGGACACGGGGCTGTGAGGCGCTCTCGGTGTGTGACAGCTG  
GGGGGGGGCACTCTTAACGAGGGTGGGCGTGCCAGGTGACTGACCACA  
GCCCTTTCCTCTCTCAAAAACGCCCGCCGAGTGACCTCACGGGAGGCAG  
GGCCAGGAACCCCAACCAACAGAAATCA

Contig 105 (1820 bp)

AGTGAGCCCTGCAGGACAGTCTGCTGAGGGGTGTCTGGGCTCCTCAGAGG  
CTCATGGCCACGGGCACTGGGAGGATAGCAGGTGGACCCCTGCATCCAGG  
TCCCAGGTCCCAGGTCCCAGACCCCGGACAGGCTTTCTATCTGCAGGAG  
GGGGGCTCCTGGGGCAGCAGGGATGTGGCTGTGAGGCCCTCGTCACTCTCC  
CTGTTTCTATCTCTCTGTATCACACACACACACACACACACACACA  
CACACACACACGCACGCACGCACACACACAGAGGCGTGACCAGGGCTGCA  
GACAGGGCCATGGGAGGACTGCCCGCAGTGCACCCAGATGGCCACACGG  
TGGGGCCCTCGTCCCACTTTTGCTGCTGATGCTTCCGCCCAGGCTGCTGG  
GAGCAAGCACTAGCTTCCCAGGGCTTGACCAGAGAGGGATGGGAGGGGT  
CATGGGTCAACAGGCGCCAGGGAATGGGGAATAGGATCTGAGGGGCGGGG  
GCAAGGGGGCCAGGCGAGGCTGCAGTGCCAGAGCTCCCTGCACCTGCAG  
GACCAGCCACAGGCCAACAGCTGCAGGCAGAGCAGGGCTGCTCCTGTCCC  
CAGAAGCTGGCACAGCACATGGGGTCTGACAGCCCCACCCCGGGCCTCCC  
ACAGAGGGGCGGGTCCCCCAAACCTCCTCCCCGTCCCACCTCACAGCTCA  
GCATCTCCACTGCCTGAGGACGAGCCCAACACACGGGCACACACACAT  
GCACGCACACACATGAATGCACCTGCAAGCACACACTCACACGTAAGCAG  
GTACACACATGCATGCACACAATGAACACACATGCACGCACACACGCATG  
CACACACGCACACACACTCAAACACGTACATGCAAGCACATGCTGGTCCT  
TTGTCCCCGTGGAGGGGAGGATGGAGGCCAGCCGTTGGGGAGGGCATGT  
GGAGTGTGGGGGGCTGGCTCCAACGCCCTCGCTCAACAGGCACCAACGC  
TGGACTGAGATAAGCCGGGGCGCTGGCTCCCTTGGGGCCGCTCAGCAGGT  
TTGACGCCCCACAGGTGGCACTGCCTCTTTCAGAAGACGGATGTGGCC  
ATGCCACCCCTCACAGCCTCACCAGTCCCCCTCAGCTTTAGTGGTGTCCC  
TGTCAGTGTACCCGGGGCCTTCCCTTCTTCCAGGGCCAAAAGCGAGTTTCA  
GGGACAGTGGCGCCCCATAATTACTCACCCAGGGTGTGTCTCTGTGG  
TGGCCTTGAGGCCAAGGTGCTCCCATGGGGGCCACAGGGCTGGCAGGGT  
CACTTCCTGAGAGCACCCAGGGCCAGGGGGTGGCCAGGCCTGGCCGGT



FIGURE 6, CONTD.

CCCACTGAACAAGGCCAGGGATTGAGCCCGCATCCTCATGGATGCCAGTC  
AGTTTCGTGACCGCTGAGCCATGAAGGGAACCTCCAATAATGCACCAATT  
TTAAATGAAAAAGACAAAGCATCCAGCCACAGCCTGAGTAAGGAGTTTG  
GAGGCCCTGACCCCTGCGTGGTCTGGGCTGGGCTGGTGGGGT  
GGGGGGGGTGGGGGGACCTGTGGACCCTCCCTCCTCAGCCAGGCCTG  
CCCCCTCATCCCTAGCTGTGGGGGCTCGGAGGAAGCGGGTGGATGACG  
GTCCCTGGGACCCCTCCTCATATGTATCTGGGTCCCTGGTCCCTCTGAGG  
CCCAGGTCAGGTCATGGGAGTCAAAGGTCAGCCAAGGGGTAGCCCAGAG  
Contig 109 (950 bp)

TAACCCACTGACCGAGGCCAGGGATCAAACCTGCAACCTCATGCTTCCTA  
GTCGGTTCCGTAACCACTGCGCCACAACGGGAACCTCTTGTCTTTGTTT  
TTAGGATTTACATACAGTGATAACGTGCCGTATTTATCTTCTCATCT  
GAATTATTTCACTTAGCCTAAGCCCTTCAGGGTCCATCCATGGTGTGGG  
AGTGGCAGGATTTGCTTCTTTTTTTTTTTTTTTTGTGGCTGAAATCAG  
TCCAGGATTATCTTCTTTCTGTTTCATCTGTGGAGGACACAGGCTGCGT  
CCGTGTGACGCTCTGCCGGAATACGGGGGCCGATCGCTTCTGAGCCAG  
TGTTCTCATTTTCTGGGAGAAGTACCCGGAGTGGAACGGCTGGGTGCTC  
CTGCAGTTCTGTGCTGCATTTTTTGAAGACGCTCGGAGCGCTTCCACAG  
TGGCTGCACCGACTGACATTTCCACCGAAGTGACAGGATTTCCCCATCCT  
TTTTCCACGTTTTCCCGCACTTGCTATTTTTGCCCTGTGGATGTGCGCC  
TCTCCGTGACGTTGTGAGGGAGTCTCCGTGCGGCCAGGCGAGGAGCGAC  
CGTGAGCGTCGTTTACGTTTCTGTGGGCCACCTGCGTGGCTTCTCCGG  
AAAAAGGGCTGTTGAGGCTTCTTGCCCATTTCTCAGTCTGATTGTTGGG  
GGGTTTGCTGTGAGTTGTGTGAGTTCGCGACGTATGGGGGGCATCAACC  
CTTTATCAGCTATGCGATTGGCAAGTCCGTTTCTCCATGTTCCGCCGGCC  
GCCTTGGCACGTTGTGGGCGGTCTCCTTGGCTCTTCTTGGTGCAGAAGGC  
TTCGGTCTGATGTGGGCCCATTTGTTTATCTTCTTTCTTCTCACCCT  
TGTTTTGATGTGAGATGCAAAAATCCATTGCCAGGGTCTGTGCCGAGAAC  
Contig 110 (306 bp)

CGCCACCTCAATCGCCGGTTTGTCTGCAACACGGTCCAGATAACCAGCG  
CACCTAACAGGTGCAACACTGCCAGAAGTGCAGACGCGGGCTGAAGCCG  
ATGGTGTGAGCCAGTGACCCGACAACCAGCGCAACAGCGTACTTGCCAG  
CCATGCGGACATCCCGGTTAAACCGTTTGCCGTTGCCACTTCGTTACGAC  
CAAACACATCGGAAGAGAGCGTAATCAGCGCGCCAGACAGTGCCTGGTGG  
GCAAAACCACCGATACACAGCAGCATAATTGCGACATACGGGTTGGTGAA  
CAGGCC

Contig 111 (800 bp)

GTTTTCCATGATGCACCAGGGGGCCGGGACCGCAGCAGGGAAGGCTCCA  
TCTTGGCTCTGTAAGACCTTGAAAACACCTCATTCCTCTGCTTGGCCT  
GCTCTTCGGTACGCCAAGTTGCTGAGACTGATGTGGGGATCAGTGGGGAG  
CAGGAATCTTTCTGATTACAGCCGTTTCAAAGTGTCCCAAGCAGAAGCTGT  
GATGGCAATGCCAAGGCTATCCATGGAGGTGGCTGTGCCAGGGGCCCCAT  
TTCCTGGGAGCCCATTCAGGAAAGGAATCTTGTAGCCCCAGGCTCCAGC  
AGCCAGTGCACGGCCCTGGGACTATCCGGGTAGATCAGAGGGAGGAACA  
GAGCTGTGGATGGTAAGCAGGTGGCCCAAGTCCAATTTATGTCTGTGGTC  
CCAGCAGGGTGCCAGGAGGCCCTCGTAACCTCTTAAGAATCTTGGTCTG  
GTCAGCTAAATTGTATGACCATTGTACTGAGCACACATCCCGTTTAAGTA  
GAATTTTCAAGGATGACTAGGAGTTTGCCACCTGAAGGCAGGAAGGGCAT  
TCCAGGCAGAGGGTACAGAGGTGAGAGGGAGGCTCTGACACTTTGGGCGT  
GCAGGGGTTTGTGTTGCTGCTGAGTGGCAGCTGGCAGCAGTGTATGCCAGGCCT  
GGCAGCGCTGTGTTGGTGTGTTGGAGAGGAAGGGAGAGGTGAGTTGAGCCC  
AAGGTCTTCCAGGCCAAAAGACTGAAGGTGACCGCGGCTGTCCGGGGCTG  
GCCCCAGACCAGGAGGGAGCAGGTGGGAGCTGGCTCTTGTTCGGGGAC  
Contig 112 (3062 bp)

CACACCCAGGAGAGGAAAGACCCACACAGTCTGATGACAGCTTGGCTC  
GGGGCTGGAGCCCCGAGTTATAAATGTCCATCACGAGCTGTGTTCTGTCA  
GAGCCATCAGTGGGAAGGCCAGGCCAGCTCAGCAGCCCCAAAATGAAGAG  
CTAGGTCTGGGATTGGGCCCAAGCAGAGGGCACAGGAAAGCCACATAAAC  
AAGGCACCAACCCCCCTGTATCCACCAATGTACATTACAGGTACACCC  
CCTGGTCTTCGGGGGAGGTCCCCTAAGATCCGGTGGCAGGGGGAGGAAAA  
GTCTGACTGGATTCTTGACAGGTGTATCAGCGGAAGGCCAGGAGGAGTG  
CTCGGGCACTGCCACCTCCAGGGGCATGATGGTCATGGACCAGATGGCA  
GTTATGGGAGGAACCTCCCCGTGGTCAGAGCTCTGGGTGTACCTGG  
TCATGCATTTGAGTGGAAGGAAAAGAAAACATACAACCTCCACCCCCAGC



FIGURE 6, CONTD.

AGCTTTAGGCTGTTGGTCTAAAGGTCCTGCCTCCTGGAAGAGACACGCCT  
CTGTGACGGGACACTGCTAAACCTAAAGGAAGAAGTGCACCTGGTCACG  
GGACTTCTTAGGCCAACCAACCTACAGGTGACGGCCCGGAGCATCACGAG  
GAGGTAGGGGACGGGAAGGGATGCATTTGCTGCTCAGCGGATCCACTGGG  
CGCTTTCTGGAGCCCCACGCCACACTTTACTGCAAATGCACAAGCCCC  
AGGCAGCAGGACAAGTCACAGTAGCTCTGGGTATCCAAGGAGTCAGGGA  
CCTACCTGGAAGAGTCTAGAACAGGTGACAGAGGAGGAGAGGATGGTAC  
CAGCAGTATAGGGAGAATCAGAAATCTGACCCACCCCTGGGGGCTGACTG  
ACTCCCAGACCAAATGCCACACTCAGGTTCCCCGCTGCTGCACTTCCA  
GGGCTGGGGCCACGGGAGTTATGGGCCCCAGGTAGCATCAGAGGCTCCAG  
GTACAGGCACAAGCAGCAACCACAGGAGGGATCCAGGCCAGGGAGCATCC  
AAGAAGCAGCAGAAGCTCCACCTTAGGTACAGTTCTGGCACCTCCAAGTT  
GAGAACATGTCTTAGCAGTGCCTGACCCCAACCAATGGAGTGTCTGGG  
ACTAGACTAGGCACGCCATTTTGGTCCCAGGTTGCCCCATCTGTACAAAG  
GGTGTGCGGGCCCCAGGGGGACACAATGAGCTCCCATGGGAAGGGTCTTG  
CGAATCTCCTTAGAAGCAGATGTAAGAGGTGACGTCCAGCTTGTGCCTGG  
GATGTAGAAGTGGAAAAAGCACCCCTCCCCGACAAGGATGAAAGCAAGA  
GGCACAAAACCAACCTGAAATTTCCAACGCCCTGGAGATCCTTGGAGAAC  
TGGGATTCTCCACCTGTAGGGGCACCTGTGAGGAGAGGCTGTGTGAGCAC  
CTGCTGACCTGGCACAGAGGATGCCCAATACTAAGAAGCATCAGCTAAAA  
GTCTCCAGGAATTCCTGGAAGCTGAGGAAGGGCTCAGGAGAGGGTACAGA  
AGCCCTGGGGCTATAGATATAAGGGACGTGCACACCCACTTGCAGGTCCC  
CATGGACCCAGGGACATTACAGTGTGGGCAAGATTCCCAAAATGCAC  
CCCTTGTGTGGGCCCTGGTTCGGTGGGTGAGCAGACACACACCAAGG  
CACAAAGCACACCCCTCAGGCTACTCTCCTCCCTCTCCCTTGTGGAACA  
TGAGCCTTGAGATGCTGGGGCAGGTGAAAAACACTGTACACTTAGGTCC  
TGGTGAAGAACTGACTGCGGCCAGCGGAAAGAATCATAAAGACCCCTACACC  
CACACACAGCCTTAATTACAGTGTGAGTGGGGCTGGAGCCCCAAGAATG  
TCTACACCCATAAAGACATAGCGTTAATCAGAAAAACAAGAACAGCCCCAA  
CCCCACCACCAGGCTGACAATAACAGGTGATGTTGGAATATCACTGGGA  
ATGTTCTAGGAGTGTAGAAAGACACACCAACTAGGGCATGATGCAAAGAT  
AATACTTCAGCCTGGGAGTGGATGTGACACAGGGAAGCATAAAGTGAT  
GGCAGAGGACTTTGATGTGAGTGTGGAAGCCAAAAAATTTCTAGCTTA  
GCTCCATTTCCCAACAAGATTGACTGCAAACCCCATGCTAAAACAACAGCA  
AAAAGAAAGAATCCTCATTTCCAGGCATAAAATTTTCCCCCAGTCTCTG  
CTGTCTCCATAAGATGTCTGATTTCAACAGGAATTACGAGGCTATAAGA  
AAGGCAAGAAAAAACTACACACTGTCAAGAGAAAGCCATCAGAAATAACCA  
GACTCGTAGCACAGACACTGGAATTGTGAGGATATTTTAAATAACCGTGA  
CAAATACATTAAAGATTCTAATGAGAAGGGGTAGACATGTAAGATCACA  
TAGATTTTCAGCAAAGAGATGAAACTCGAAGGAAAAATTAATGGGAGCCCT  
AGAGTGAAAAACACTGTAGCAGAGAAGATGGGTTTCATCCGTAAACATGAC  
ACAGCTTAGGAAAGAATCAGTGAAGTGAAGACAGGGCCACAGAAAATAT  
CCAAACTGAAATGCAAGGAGGAAAAATATGAAAGGGGGAGAGAGAAAA  
ATAAAAGAACAAAGCATCCAAGAGCTGGAGGGTGACACTGAAGAAGAGAG  
CATAGGCATAGCTGGAATCTCAGAAAGAGAGAAAGAAATAACCAAGATG  
TAATGGATGAGAATTTACAGAAAGCGTTGTCAAGCAACAAACCATACATC  
CAAGAAGCTCAGAGAACACCAAGCAAGTAAGTACTGTAAAAAATAGCC  
CGAGGTATACCTCATTCAGGCTGCTGAAAATCCATGACAAAAGAAGTCTT  
GAAAGTAGCCAGAAACAGAAGGCGTGTTCATTGAGAGGGAAAAAGACACC  
ATTGTTGCCAGAAACCAATAAACCAGGGCTGAAAGGGTAAAACTTTTTT  
TTTTTTTTTTTTTTTTTGGCCATGCTGTGGCATGTGGAGGTTTCCCGA  
TCAGGGATCAAC

Contig 113 (1300 bp)

AAACGGATAAATACAGGTGACCCACAGGCAGAAGCTGAAGTACAAACAGT  
TCACAACGGCACCCAAAAAATACCGAAGGCTCAAGGGTAAATCTGACCCC  
AGATGAAAGGCCTTCTCACGGAATGGCAAAGTGGCGCTGAGAGGCATG  
AGAGGTTGCAATAGATGGAGGGCTCCGCCGTTTTCCCGGGTCCGAGGATT  
CAGTGACGTACGACGCCAATTCCTCTGAAACGCCTCTCTAGGTTTCAAGT  
CAGCCCAGACCCACTGGCAGCCGCCCTCGCTGCAGAGACAGCCAGCTGG  
GTCTTGAGGTTCTACAGCGAAGCAAGGGTCTAGAAAAAGCAGACGTCT  
CTGGAAGGGGAGAAGCAGCCGATGGATTGGCATAACGGGACAGGAGATT  
CTCGGACAGTGGCACCAGGAGAGGGGTGGACAGAGACTGGTGAACCGAG  
CGGGCCCAGGAATAAGTCCACACCCACACGTACCATCTCGTTGTTTATTT  
ATTTTTTCTTTTTTTCAGGGCCACTCTGGGGCATGTGGAGGCTCCCCAGCC

FIGURE 6, CONTD.

AGGAGTCGAATCGGAGCTGCAGCTACAAGCCTACCCACAGCCACAGCGA  
CACAGGATCTGAGCCATGTCTGCAGCCTACACCACAGCTCCCGGCAATAT  
TGGATCCTTAACCCACTGAGCAAGGCCAGGGACTGAACCCACGTGCTCAT  
GGATACTAGTTGGGTTTGTACCACTGAGTCACAGTGGGAACCTCTTTAA  
TTTTAATTTTGAAGGTTTCAAACTCTTTAATTTTTAGTGAGGTATAGA  
TTATATTACGCACCATTTCTTTCTGACTTCGGTGCACGGCTTTTCAACAA  
ATGGGTGCTGGACCTGCTGGGTGCCTTCTTCAAATGAACCACAAGCCCTC  
CCTCGCGCCGTATGCAAAATTTAACTCGAGGGGCTCATAGACATAAACGT  
AAACTCTAAAGCTATAAAATTTCCAGAAGAAAACGTAAGGAAAACCTTTG  
GGGTCTTGGGCAGGATTTCTTACCCATGACAGCAAAATTACAATCTACA  
GAAGAACTGGTGGCCTTTATCGGCATTTAAACACCTGCCCTTTGAATGA  
TGCTGTGCGCAAAACCGAACATGCAGCAAAACGGATGCAACTAGCAGGTCT  
CACACTCAGTGACCCACGTGAGAAAGGAAAGACAGCCACGTGACATCC  
CTTAGATGCAGAATGTAAACACGGCCCCCGTGAACCGACCTCAAGAGAG  
AGACAGACCTACAGACGCAGCAAATTTGGGGTTGCCGAGGGGATGCCGG  
Contig 114 (3000 bp)  
TGTGAGACCCCTTGGCGGGCCAGGACCCCCAAGGTGACCGAAGGCCTCA  
GCGCCCCCAGCGCCCCCATCCCOCTCTTTCCCGACACAGGATTTTTTCC  
CACCAAGCTCTGTTCCCTTGGTCAAGCTCTCACTTGAGCAGCCTCAGGGT  
CTCCCGGTGCCTGTATCCACGACAGCGTGACCTTCTTGGTGTGTAACCC  
AGGACCCACAGCTGGCCAGCCAGCCTTCCAGAGCACCCCGCCCATCC  
TCAGAGTCCAGAGGAAAGGCCCATTTGACCCAGAAACAAAACGCAGA  
GACTCTGGGACGCCAGCAAGAACGTACTGACTCCACCTGCTTCAGGC  
ACGGAGGCAGGGGTGGGTATGAGCGACCCCGTGAAGGGCCTTCTTGTG  
CATCGAGGGGCTTCCAGGGGCTCCTAGACGGGGATGAGTGTGGCAACATG  
TCGCCGCATTACAAAAGACCTGCAGTCTGCTGGGATGGGTCCCCGGC  
TAGAAAAGCAAAGGATTCAGCCAGTTCGAGTAGGAGGCGGCTCGGAGG  
CTGCAGAGGCGCGGGGGCGCTGACCACCACTCGGCAAGCCCCGTGTTGG  
AGGGGACGCCCCGGCCCGCTGCAGCCGCTGCGCCTCCGGATAAGCTCCTA  
AGAGGCCGCGTGCCCCATGCACGCGCGTGACACACTCGCTGCCCGAGGG  
TCCTTCAGCACAGACCTTGTGGGGACGGAGGACCTGGCAGGGGTGTGGCT  
CTGGGGAAGGGGTCTGTCCAGGAACCTGTTCTGGATTGGGGGTGGGC  
GTGGATATCCCGTCCCAACCTACAGAAGGGAGGGGCTTAAAAAGAGCCCC  
TTTGGTGTGAGGGGCCAGCAATCCTTTGGCTTTTTCTTGGCCACTTGA  
GCTTGACGTCTGGTCACTGACTGGGAGCCAGGGCCAGAGGGGGCAGCCG  
GGCTGAGGCAGGTTTCAAGCCAACCATCTCTCGCCCACTCCCGAGGTG  
GGCAGCTACGGGGCCCCAGAGACACAAGCCCCAGGGGTCTTCCCCCCC  
GCCCCCTGCCCCAGATCACCAGGAGACCCAAGCAGCTCTGCCTCCCCGTG  
CCTGAGAAATGCCCATCTGGGTACCAAATCACCTCCAGAAAGGTAGA  
GTGGGGGGCCAGGACAGGGGGACCCAGTTACAGAGCCCCAGGCAGGCT  
TCCCAGGGGCGAGGGGACTCCGTTTGGGGCACAGACGGAGGCAGAGCGGG  
CTGATGGATTCTCCCCCGGTTCAAGGATGCTGGCTGCCTGGCCTCCAGGA  
GCCGGCGGTGCCATCTGATCTGATTAAGGCCTGCAGTCCAGCTGGGCGG  
GCACAGCCTGGGGGCTCGGCGGGCAGGGAAGAAGCGCTGTGCCCCAGC  
CGGTCAAGCTCGCTTTCTCTTCAATTCCTCTCCATTAAAAGTGTGAAAC  
CATTTATTGATTTTTTAAATCAGGACGTGCTGTCCGTGACACAGCAAAGT  
GAACAAAATCAGAGCAAAGAGAGGCCAGGGCTGAAGCCCCAGAGGGCGGC  
GCCTCCAATCCGGGTTGTGCCCGGGGCTCCAAGCCCTTCTTCTCTGG  
GGTCTTGGCGTAGTGGCCAGGGCAGAATGCACCTGCCGTATCTTGGGA  
GGCTTGGCCATCGCTGGCTTCTGTCTCATGACGCACCGTCTGTTCCATATC  
TACGGAACAGCTTCGCATTAACAGGCAGGGGAGGCGGTTGTTTCTCCTT  
TATCTGCCACCATCGGCGCTGGGGCCACGTGGAGCCAGCCGGCTGACT  
TCCCGCTCGCAGCAGGGGACTGATTGCAGGAACGAGGACATCCAGCCCC  
CGCCTCTCAATGCCCCGGGTGCTGAGAGCATTTGCCCCAAACGGCTTGGG  
TGGGACAAGGATGGAGCTGTGCGCCAGGGGCTGGCTGGGGCAGAAGGG  
GGCCTGCCCGTGTCTGCCCGTGGCCTCCAGCACCCCTCGGCTGCCAGGCTG  
CTCTGGAGAGGTGCCCGGGGGCCAGGGCCAGGGGCACCCTGTTCTGCCC  
CACGTCTCTGTCTGTGCTGAAAGTTCCACCAGACGCGTGTATACCCTG  
GGAGTCAGGAGGATGGGGGATAGTTGGGGCTTGACGTCTGTTTCTGAAAA  
AACACCGTTTTCCCTGAAATATATATGTATTAATTTTTCTGCAAGATAAA  
ACTGTGTATAGTTTTTTCGTGATGAGAAAACGCATCCATCTTCTTAGAAA  
GCCTGAAGAGGTACAGGAGCCTATAAAGGACAAGATGACAGATGCCTCTA  
ACGCACACCAAATGTGCGGTGGCCCCCAGGGGACCGCATAGACGGGGCGG  
CTCCAGATGGCCACCGTGTGCGAGGGACACGTTTCAAGGTGGCAGAGTAT

FIGURE 6, CONTD.

TCCTGGGGGGGGGGGCTCAGCGGTTCCCATTTCCCCCTCCCTTCCTTCC  
TTCATTTCTTTCCTTCTTTCTTTCTTTTGTGGTTTTAGGGCCGCACCCG  
CGGCGTGTGGAGGTTCCAGCCTAGGGGTCTAATCAGAGCTACAGCTGCC  
GGCCTCCACCACAGCTCAGGCAACGCCGGATCCTTAACCCACGGAGCGA  
GACCAGGGATGGAACCTGGGACCTCATGGATCTTAGTTGGGTTTGTTCCT  
GCTGAGCCACAACGGGAACCTCAGCCATTTCCCATTTCTTGCTCCAGTTCC  
AAGAATTTCCAATTCTTATTCCTGTTCTTTAAGGCCAGAGGGCAGACCCAC  
GCCGAGTCCCAGAAGCAGGGCTCAAGGATGCTGCTGTTGACTGTGTCCGT  
GGGCGGGGGGAGTTGATAAGAACCCCAACACAGGGTGTTGGCCAGCAAC  
GGGGGAGGGAGGAGGGGGGCTGGTGGGGAAAAGTCCCCTGAACCCCATGG  
GCTGCCCCCTCCAGGCTGGGGCAGACCCCGAGCCCCATGGCCCGAGGAG  
AAACGGTCCCAGCCCCAGGCTGGGCTCCCGCACCCCTGCCCTGACCCCGC  
Contig 115 (1895 bp)  
TCATGGAAGCCCTTATCACAACTCGGATCCAAAACCCACTGCGCGAGTC  
CAGGGATAGAACTCGCATCCCCACAGACCCATGTTGGGGTCTTAACCAG  
CTGAGCCACATGGAACTGGGTAATCTATTTTAGATGTTCTTAGGGTTT  
TTGGCCTTGCTGTACGTGGGGACGCTGCTGGGCCAGGGATCAAACCCGC  
GCCACAGCTGTGACCCAAGCAGAGCAGTGACAGCACCCGGATCCTTAAGCA  
CGAGGCCAGCAGGGAGCCCTGTGTTTAGATTTTGGTGAGGATACTGCGT  
GGGATTCAGGATATTCACCTTGGGGCTGTGGAATTGCCCGTGCCTGTTT  
AAGCAAAGAGAAATCCCTTCACTCTGTGTAAGTGTGGGGAAATCCTTTAG  
TCTCTTGAACCATTTGCGTGTGTTAAGAGTGGTAAGTCTGCCACCATAA  
ATGCCCAGACCAGCGCCTTCTGAGATCCGCTTTTGTGCAATATCTGG  
TTTGAATGCTTTGATCGCCCGCACCAGACCAGGGTGGGCGGACGCCGCCG  
GGGACCCGACGTGACCATCGTGCTTCTGTATCCGCCCTTTCTCCGGCAGC  
CGCCCCCTGGTTGCCTCTGGCTGCTTTTAGTGGAGGAAGTGAAGCCTCGC  
CACCCAGACCCCGAGACCCGAGGACCCACAATGCTTCAAACACCTGCCCT  
CTGACTTTTACAGGTCAAGTTCGCCAACGCCGAATTTGCACCGATTGGCT  
ACAGAGAGCACGGTGGCGCCAAGCCTCCACTTGGAGTTTATAAGGTCTC  
CCTCCAGCTCGCAATGAAAATGAGCTGTGATAAGGCAAAGACAAAATTAG  
TATGAAATCCAGATGCTTCATCTACAATACAATGACCGCGGATTGCGGT  
CTGAGCGACTGAAATCAAGGTGGGCTTCCGGAGGGAGGCTGTAGAGGAA  
AGGCATTACGGAGGCTCAGGTCCGAGAGGCTTCCACACCCCTAAGAGGG  
CTGAGACGGCAAGTAGGGACCAAGCCCCGAGTCGGGAGAGCTGGGCAGG  
AAGGAAGTCTGAGGTCACCCCCACCTGGGGAGGAACTGCCTAGAGAAGCG  
GGGGCGGGAAGCAGGGGATGCCAGTCCCAAGACAGGGACAGGGCGGAAA  
GGGCTCTCTGCAGGCCCTCAATGCTGCCACAGTGTCTCGTAAGAGGGAG  
GCAGAGAGAATTGACACCGGGGAGACCAGGGACCACGGAGGTGGAGACC  
GGGCTGCCCGCGCGTGCAGTTGCTCCCGAAGCCGGCCCCCTCCCCCAGAG  
CCTTTGGGAAGAGGCGCCAACCTGCAGTTCTGCTACTCGGGGACAGGGAC  
AGGGACAGCCCCCTGGAGCCGCTCTTAGGGGAGCATCCCCCAGAACCT  
TCCTTAACAGACCATCTGGAGAGAGATGGGTCTGGGCTGCAGCTCCTGGA  
ACTGTTTGGCCACCCGGCGAGCACCAGTGGGTGCCAGCCTGGGCTGCCC  
AGCCTCAGGGCCGGGAGGGCTGAGGGCACTGGGGCCCGGCTCTGGGACT  
CCCCTGCTCCTGCCCGTGAGGACAGCCACCTCCAGCATCTGCTTCCT  
GCCACCCACATCCCCAGGACCGTCAGCCAGGCATGCCCTGGCGTCGGC  
CACTCACACCACAGGCCAGGAACCCAAGGGGGCAACACAGAAGGGCAGTT  
GCCATCTGCAGATGGAATGGACAAACTGGGGTCCGTGATGATGGCAGGCT  
CTGGGCGCCCCGGGTGGCAGGGGAGCCAGGACTGTGCGGCCATCACAGGA  
AGGGCATGACGGGGTGAAAGCAAGAGTGGAAACCTCTGCCACCCGCTTG  
CGCACATACCGGCCACCCTGCAGCCCCACCCCATTTGTTTGCT

FIGURE 7

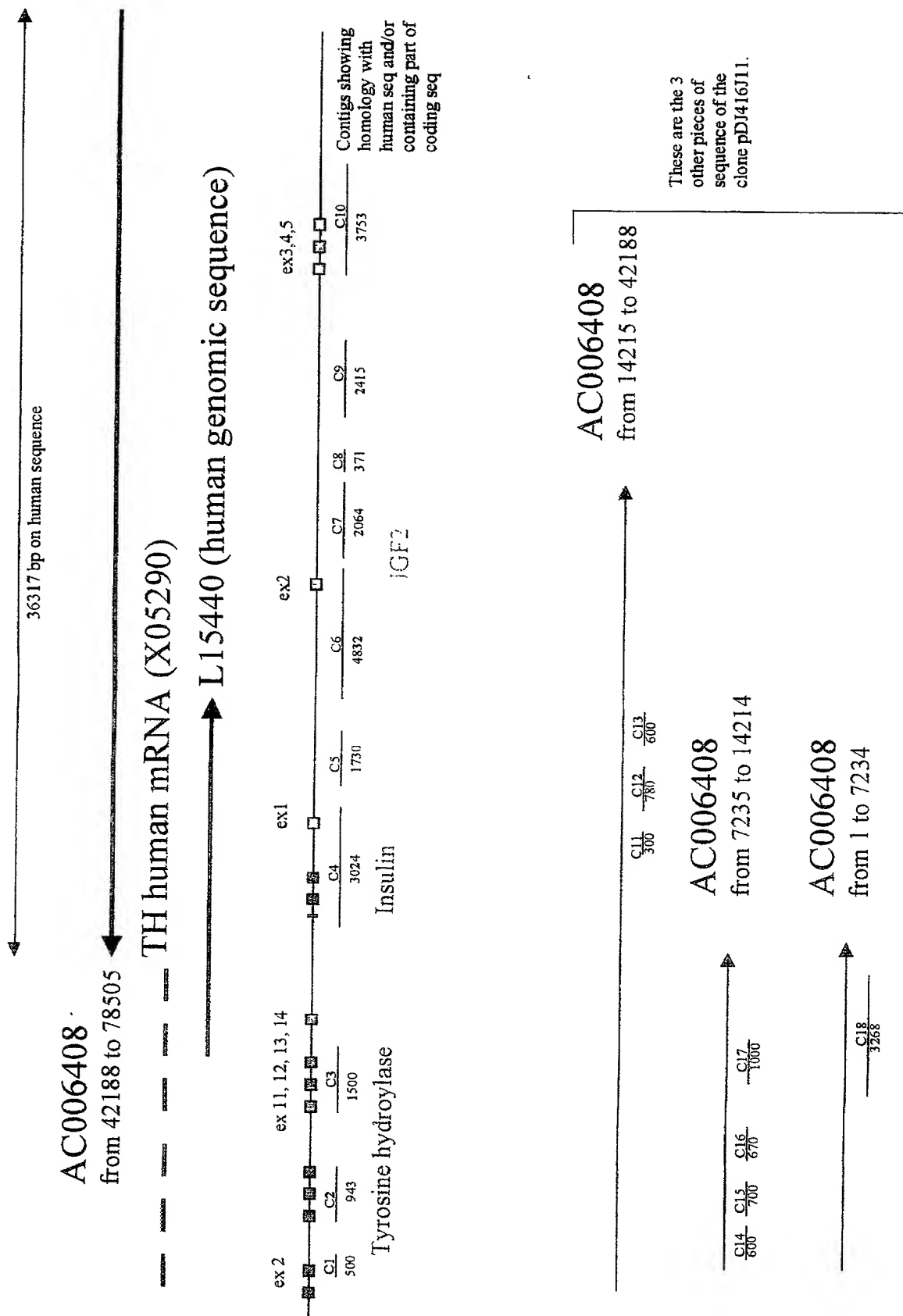


FIGURE 8

## Contig 1 (1040 bp)

GCGCGCCGGATCCTTAATTAAGTCTGAGAGATCTGCGGCCGCGGCCAGGGTCTGCTTCTG  
GCCAAGTGTGGGGCTCTGCTCCATCCTGGCTCGGAGGTCCACCCATGGCAAAGCCTGGGG  
TCCTCCCACTGAATATTTGGGGGTCCACTCGTGCCAAAGGCTGGGTGTCCAGTGTGCCAA  
CGGTACATGGAAGCAATGTCTTCCCAAGGACCGTCCAAGGTGTGGTCAGGCCTGGACAGC  
TGTGAGTCCCTTCGGGACTAGACTTGGTGGCCGAACCCTAGGGACCGTGCCCGAGGGCCC  
CCACGAGGCCAGGTGTTTGGCCCAAGGACAGAACGGCCAAGGGTGGCCGAGGGTCTTTTT  
TGTTTGTCTTTCTCTTTCTCTTTCTTTTGGCCGAGGGTCTTAAAGCGCTCTCTCTG  
CTCTTTGTCCCGATCCTGAGCGGGCAGTGTCTTGGTTCGGTGGGGTGTGGGCAGCCGAG  
CAGGGCTGAGAGAGCCCGCTTGTCACTAGGGCGCGCCGGTGAAGCCAGCGGGCATGCCG  
TGTCAGACGTTGGATGGGGCAGCGAGGGGACTGGGGTGCCCGAGCCCCCTGGGAAGCC  
CGCCCTGTGGAAGCCGCTGTGCTCGCCACAACAAGCACCGTCTGACTAGCTGGTGAATCAG  
CGCCCGTTCGCCCCGTAATCCAGGCGCTTTCTGCCAACCTGAGCCCTGACCCACACC  
CCTTGCGACCGCTCCGTGGACCCCTGGGGCGATGAGGTGAACCGTGGGCTTGCCATCGTG  
GTGGCAGACGTTGGCACACCCGTGCGCCTGTGCGCCCCCTCCATCCAGGAGCAGAGTGC  
GCACCCAGTGGGGCTGGGCAGGGAGCCGCTCCACCTCCGCCCTGAGGGGACGGGACTC  
TTTCGACCCGGAGTGGGAAGGGACATATGCGGACGATGCCAGACCCCTGTCTGTGGGGGA  
GGGGGAGAAGGCCCTCTTTGGAGAATTCCAGGACGGGTGAGGAACGTGTGCTGGACCGGC  
CGGGTTCGGAGGTGGGCCTTG

## Contig 2 (9234 bp)

GGCAACCAGGGGAAGATGGGGAAGCGGGGTGCAGGGGCGTTTGGCGGGGCCAAGGACCAC  
CTTGGAATCTGGAGCCTGGCAGGAGCGGCGCAGGGTTGAGGGGCTGGCTTGGGCAGGGC  
TGGCTGGCACCTGGGAGCCTGGCGGGGTTGAGGTCCGGGCTCCAGGTGCCCTATAGGCA  
GGGCAACATCGGCATGGGGGTGACAGGCCCGAGCTGGGGTGCAGGAGGAAGAGGGGGGA  
GCCAGGCATTATCCCGGTCAATTTTGGTTTTCAGTTCGTGGCGGCTGGTGGTCAGGGGGA  
GTTGGAGAGAGGTTTCCCCCGGGGCTGGGGCAGCGGAGGTGTAGCTGGCAGCTGTGGGC  
AGGTGAGGACAGCCGTCTGCGGGGCCAGGTGAGTCCCTTCCCTCCCGAGGCCTTGTTC  
TCTGGCTCCTGCATCCGGAGGTTCTGGGGAGCGAGGGCCGGCGAGGCCAAGCGGCTGAC  
CCCCCGGCAGAGTGGCGGCGGACGACAGGCAAGGCGGGCAGAACAGGTGACACGTCTCAG  
GGGGAGCTGGGACCGGGCGGGCTGGGGGGCCGGGGCCCTCCAGGTGGAAGAGCATCT  
CAAGCGAGTCTGGTGGGAGACGAGGCAGGGCTGCCAGCAGGGAGGAGACGCAACAGGCGG  
GGGGCATTCAGGCCCCGGTTCGGACAGGACCCGTGGGGGTTGTAGGACAGTGGGGTCCC  
CAGCGCCACTTACCCACTGCAATTCATTAGTAGCAGGTACAGGAGCGGCTCTGGCCG  
GGCCTCTTGAGGCCTGAGCTGGAGCCTCGAGGGCCGGAGAATGGGAAAGAAGGTGCAGTG  
TGCCAGACACAGCTACCTGAGGGAGCACGGCCGTGGGGACCGGCCCCAGAGAGATTTT  
GGCAGCAGGAGGCTGCGCGGGCCAGCCTGCGGACGTGCGTTCCACGCAGCACTGCGG  
CCCAGGGGCTGGCGCGGCAGGGCCCCCGGTGTCTTGGTGGCACTGTGCGCCCTCGCCGC  
TCGCCCCCTGGGACTGGCACGGCAGACAGGACAGCACCCAGGGGAGTCAAGGGCACTGAGC  
AGACCAGACTAGGCGAGGCGGGTGGGGTGGAAATGGATGTGACCTCTGGGGGGAGGGAGGT  
AGGGACCGAGGACAGGGGCGAGGCGCGGAGCCTGGCGGCGAGGAGGCCAAGGCGGGCCT  
CTGCGGGTGACAACCTGAGCACATATGGGTACCTTTGCGCTCGCACCGGAGACAGGTGAGT  
GTCTGCCCCCGGCTGCGGCCCTCCCGGCCCCGCACTGCGCTCTGCGCTCCCCCTCGACC  
AGGGCCCTCTGCTTCCCCACAGCCTCGTCTCCAGTGGGGGTGGACACACTGCCAGCACCA  
CAGGCCGGACGCCAGGATGTGCTTGGAGGGACATGACACAGTCCCGGTGTGACGGAGAGGG  
ACAGACGTGACGCCGTCCGGCCCTTCTGGTGAGCGCAGGTCCAGGCCTTGGCCCCCAGGC  
CAGCCGCCCCACCCCCACCCCTCATGGCCGCTTCTGTCCCCGAGAACAACCTCTGGGCTG  
GCCCCGCGGGGGAGCTGCCACACCCAGCGTCTGTTCCCTTTCCTTCTGAAGGAGCACGT  
GCATGACTGCTGCTCTCTGGACCCAGAACCCCTCAAACGACAAGGTGAGGCAGGTCCCGC  
CTCGCCCCACAGTGGGAAGGGGCGTGGGCGAGAGCCGGGCGCTCACGGTGGCCCCCTCCC  
CCTGCAGAGATGGTGTACCCAGCTCATGCCCTGGGGCTTGGACCCGGAATCTTCAAGTC  
CTCCTAGCTCTGACTCAAGAATATGCTGCATTCTGGAGCCACTACACTACTTGACTCAGG

FIGURE 8, CONTD.

[illegible]

FIGURE 8, CONTD.

AAGAGCAACGTCTGAGCTAGCTCCACGCGTGGGTCCATCTCGGCCAGGTTTAAATGAGCC  
 ACTTTCAGGCAGGGATTGCACAGGAGGCAGGGTGGGAAGTGGCTCTGCTCAGACCCCTGA  
 ACAGGGTCTGGAGATTCTCCAAGGGCACAAAAGAACGGACGATGCCCCGGGGTTCAGCGA  
 CAATGCTCCCTGAGAAATCTTGGCACACAGGGCTGGGCCTGCGAGGTGGCCCCCTCGCCCC  
 ACCCCAGCCTCCTGGAGGACAACCGTTCGCCCTGCTCCAGAGCTGGGGGGCGCCACACGT  
 GGGGCACAGGAGCATGGGCCCGATTCCAGGCTGGGCTCCCTCTCGTGTCCAGGATCTC  
 CCCGTGTCTTGTCTCAACAAGCCCTGACTTGGAGGCCCCAGGGTGACCCCTTAAAGGGG  
 GAACAGAAGGTTCTAGAAGGAGCGTGGCCAGCTTTGGCTTCCCTAGGGCTGTGGTGACCA  
 CACTGGGCCACGGCCAGGCCACCCACCCGCTCCTTCCCCCTGGCCCCCTCCCTTCCC  
 CGACCTCTCCTGGCCTGCACCTGGTGACACGGCTGGCTCCCAGCCAGGGCTGAGGGGG  
 ACCAGCGGGGGCCCTTCTTGAAGCCACCTGCAGGCCGGCTTGTGGGAAGGGGCGCTGC  
 TCCTCGCCGGGCCACCCCGCCGGGGCGTTTCTTGAAGCGGTCACTGGATATTTTGT  
 CCTTGTGACGCGGAGCTTGCATAAAGCAGACACTGAGCTCCTTGTCTCCGGGAGCACG  
 CGCTCCATCACCGAACACCTGGCCGGACACAGGCGGGCAGCCGGGGCTGGGGGAGCAGCG  
 CGGGCCTGGGGCCCGGACAGCAACGATCACGGCGCCGAGCGCAGGGCCCGCGCGCTTC  
 TGCAGGCCGCCCCACGTGCCAGGCCAGCGGTGCCCATCCTGCAGGCTGGGAGGAGGC  
 TGTGGGCGCAGAGCTGAGAAGGGGGCAGAGGCACTGGGGGGGACAGCCGTGTTCCACAC  
 CTTTGCAGAAACCTTGGCCGGCTGGATGTCTTGTGGGAGAGCTGGGGGAGGGGACAGG  
 GCAGGAAGCCGGTCCCCCGAGCGGGGTAGGAAGAGGCCTCGGCCCTGGGAGGAGGAGGA  
 GGGGAGGGCAGTGAGATGGAAGAGCACCAGGGGCTCGAGGCTTCTTTCTGGAACAAGGA  
 CTAGAAGGAGGAGGCCGGGGCAGCTGCTTGGGATGCTTGAACAGGCCGCCCCAGTGCTG  
 ACAGGGACGTGACCTGGGGGCGGTCCCGGGCCAGGCGGGCTGGGAGGGCGCCTGGTGG  
 GTGAGCGCCACTCAGAGCCCTGGCAGCAGGGGGCTGGGCACGGCTGCAGGACAGAGCTC  
 AGGACACAGATGGGGGCGAGGACTGAGTGGGGCACACAGATGCTCCAGGAGGTGGCCA  
 AGGAGTGGCCTTGGGATCCCAGGATGGCCCTGGTCCAGAAGATGCCGCAGCCCAAGGGA  
 CCAGGCCAGGGCCGAGGGGGCCACAATCTGAGCAGGGCTCAGGCCAGGGCAGAGGCC  
 CCTCCACCCAGCCCTCCCTGGGCCCGCTCTCC  
 GTGCAGGCAGTGGGCTCAGATGGGGCAGACATGAGACCAGGTCCAGGGAGAAGCGGGGGCC  
 CCTTGGCTTCATTAGGTGGCTTTAGACCGCGCCCCGTGCGTGGCAAGGCCACAGCGC  
 TCAGGAGCACACAGACCCCCACACGGGCTCCCCAGGTGGGCGGTGACATCAGCCCTG  
 TGTCAACAGCAGGAGCTGGCAGCTCCCCACGGGGCTTAGGGAGCGGGGACCCTGAGCCA  
 CCCTGCCACCGCCCCACCCACCGTGGCCACACAGAGGCCCCGTGCTCTGGGTCTGGGG  
 CCAAGGCCCCCAGGCGCTGGCACTGTCTGCCCTCCCGCTGGCTCTCCGTCTCCAGTG  
 TCCCCGCCAGAGAGCATGGGGCCACAGGCCTGAATGCCACCTCTTCTCCTCTGGAGG  
 GGGCTGAGGTTTTGGGGGTTACAGAGTGGCCTCCGGGGTGGGTCCAGGCCACGAGG  
 CAAAGCGGACCCAGGGAGTCCCGCGGAATGTGGGACAGCCCCCGTAGATCTCGGGGG  
 GGCCAAGCTCTGGTTGACCTCCATCCTGGGGCTGTGGGCCTTGGTTCAGTGGGGAGGGTC  
 ATGACACCCAGCCCACAGCTGGTGACAGCCCTGGACGTGCCGGCTCAGGGCTGGCCTGC  
 CCTTGCAGCCTTGAACCCCTGTTCTCTGGAGTGGGGGCGCAGGGGGCGCCGGGGCAGGG  
 TGAGAGACGAGAGCCTCTCTTCCAGAACTTCTGCCTGCGATGAGGACCCAGCAGGGCC  
 TCTCCTCACCAGAGGGCTCTGCCGGCTGCAGGGCCCCAGAGAGGCCAGAGGCTGGAGG  
 CCGGGCCTTGGGAAGAGGCCGGAATTCAGAAACAGCTGCCCGCTCCGCAGCACCACAGC  
 GCCCACTTGGGAGGGGGCGCGCCCCCGTGGCCCGCCGGGTCCACTGCTGGGGCCGCCA  
 CAATAAAGTTTTGTCCCTGCTGGTTACTGTCCGTGTCTGAGAGGTTTCTGGAGCCTGGCCA  
 CAATGGGCGTCAAGGATGCGGCTGGGAGGGAGCCTCGCGAGTCAGAGTGTGCTGGTCTCG  
 ACAGGCCCGGCGCCCCCAGCCCGTGTCTGTGGACAGATGGGTGGGTGGGTGGGTGTCTG  
 GAGGGGGTTGGAGAGGTTGGGCGGGACGAGGGGCTTCTGCACTCTGTCCCAGGGAAGCG  
 GGGACCAAGGAGGGGACAGCCCCCGGTACCAGGAGGGTCTGTCCCTCTCACCCCCCGG  
 GACAGGTGAGCTCCCCGGAGCCGCTTCTGGGACAGGACCCACGGCCAGGCCACGGCC  
 CCCCCACCCCGTGGTCCCTCCGTCCACGGCCGGCTGGGGGGCCACGGGCCAGGGCC  
 CCGCTCCCCGTGGCCCTCCGAGGGTGAACGACCTCGCCTGGGACGTGGGGCAGAGGGC  
 AGGCGCCAAGAGTGACCCCTGGGACAGTGGCTGTTTGCAGTTCTGGAGGCAGCCGAGA  
 TAAAGCGGCTGTTTTCCAGTGGGCTCAGGGCCAGAGGGGGGCGAGGGGCAGCCCCAGTC  
 AAGGCCGGGCGCTGCCTCGGGTCCCCCTCTGTGCGGAGGGAGGGGGCGGTTGCACAGC  
 AGCCCTGCCCCCGCCCGCCCGGCGCAGGCACCGTGGGACCCGGCCCTGGTCCCCCT  
 CCCCCGCCCTGCTCAGGGGGCAGCCCTCTCTGGTTCCAGGACGCCCCCGCCCGCAGG  
 CGGCCAGAGAGTCCAGAGTGTAGCCTCCACGTGTGGGATCCTGTATATGCGACAGC  
 TTAAGTCAAGGCGAATTTTATGGGTCTTGGATTGGGTGGGCACGGCCCTGCACAGCGG  
 GGCTGGAAGCCTAAGGCGGTGGGCGTGGGGGTGAGAGGCCCGCAGACAACAGGAGGGAGG  
 CTGGGACACTTCAAGGGTTGACATGCTATGCCTGTACGGATAAATGC

Contig 3 (5347 bp)

AGATGTGTATAAGAGACAGGGGCTGGGTGGGAAGGACAGAGGGTGGGGCCGGAGGAAATG

FIGURE 8, CONTD.

GGATGCAGAGCCCCACCGTGCACGCTCTGCTGGCCTTTGAGCCTCGCTGAGTCGCAAGAAG  
 CCCTCGGGCCTGGAAACAGACCCCCGGCCCCACCCCCACCCCGCCCCGATTACCCC  
 GGCATGGCTGGAGGGCCCCGAGAAGCCACCCAGGCTTCCCGTGCCGAGCTGGGTGCTGGGC  
 CCAGCCGAGCGGGCTTGACGCCACGCTTAGCCCTCCCCAGGGAGCCAGGGTCGGAAGGA  
 AGAGGCCGGCCGGAGGGCCGTGGCCGCTCAGGCTGGAGGGGGCCCCCGGGTCAGGATGGG  
 CCCCAGACGTCCCCGCTCCCCGGCCATCCGTCACGGAGCTGTACCCAGGAACGTGCTCC  
 AGACGTGCTTTCTGCGCGCGAGCCCCGAGCAGGCTCCAGGCGCCCCACCCCCGAACG  
 CCCACGCACACCTCGGTCTGCGAACACCTGCCGTGTCATCCGGTGGCCCCGGTTCCCGCC  
 GCGCGCGCATCCGGGTGCCCCCTTCCCTCCCTGGGTGGGGGGCCATGCCCTCAGCGGGCAC  
 GCAGGCCTGTGAGGTCTGTTCTGACTCTTCCCCAAGACGCAGGCGGCTGCGGGCGCC  
 CCGACCTCGTCTGAGGCCCGTTTGTGCTCACTGGCTGTCTCAGAAAGGGGTGCCACGGG  
 AAGCGCGTGTTCCTTGGGCCCGCAAGGCAAGGGAGCCACCCCAAGGTGGCTGAGGGCAAA  
 TGGCCAGGGCCTTAAGGAGTCCCTGGGGGCCGGGCGGCGCTGCAGCTTGAGGAGGAGA  
 GCCCTGGCTCTGCTCCCCCGGGCAGGTGAGCCACGGCAGGGGGCTCCCCAGCAGCCTTG  
 GCAGGAAGCACTGAGGAAGGGGTGAGGATGAAGCAAGGGGGCTGCGGGGACTTGGGCA  
 AAGCCCCGAAGAACTGAGTTCTCGGAAAGGCCGGAGCCCTCAGCCGAGCCTCGGCCCTC  
 CGAGCGATGGAGCGGGCCACCTGCGGGCCCCAGGGTGCAGCTGTGCATCCGTCCCCCTCG  
 GGCTCCCCCTGCCCCCGGCCACCACACTCTCCCCCTTTTGCTTTGATCACTTGA  
 GCGACAGCTTGTGCGGCCGTGAGCCCCAGAGACCGCTGCCCCCTGCCGCCAGCCCCACGG  
 GAGCGTCCACCTGGGCCTGGCTGGGCACTCATCCCTCCCGATGAGGCCTTTCTAGCCT  
 GGGCCGCCCCGGGAGCGGCAGACCCAGCCCTCGCCCCCTCCCCAGTGAAGGTGCTGC  
 CTGGTGGTCTGGGGAAGCCCTGGAACAGGGGGCGCAGGTCCACACGGGTGCTCTGGCC  
 TCCAGTGCAGGGAGGGCGCGCTCAGGCCAGGGTCCCCCTCCACCAGAACCGCCAGGGC  
 CCTGGGAAAACCTGTCTGTGCTAACAGGGCCGCTCCCCGGGACTCCACGAGAGGTGCG  
 AGGGACCCCTGAGCACCCACGCCACTAAGGGGGCCAGCCAGCTCGCGGGTGCAGGCAGC  
 CGGCTGGGCGCTCACATGCTACTGCTCTCTGGCTTTGTGTGTGCGCCTGGGTTGGGGTG  
 AGCGGAGGTGCCCGAAGGCGGAAGAGCCACCCCTCCACTCGGGGACCTATTTACGAAGA  
 AGACGGATGGGACTGCCGGGCATGGACAAGGAACAGGATGAACCTTCTGGAACGCACAA  
 GGCTTCCACGGCTGACCGGTGATAGGAAGGCGGTCTCTAGGCCAATCCACCGTCCACCG  
 TCCATTTCCCGAGCCCTCGAGAGGGGGCAGGATGGACCGCTGCAGCGTGAGAGAGCTCTGG  
 GCGCTCCACAGGGCAAGTCCAGGGCACTGACCTCAGAGCCCAACCAGGCCACCGGG  
 GCTGGGCCCCACAGGGAGCCGGGGCCAGGGTCAAGGTGAGGGCCAGAGTGCAGGAAAGG  
 GTGGCGTGTGCTTGGGGCGGGCGGCAGACGGCCCTCGCACCCCCGACAGCCCT  
 GGAGCTGAGTGAAGCCCGGGGTACCTTGGCTGGGGTGGGGTCTCTGCGACCGGCAC  
 CCGAGCTCAGGTGATCTTGTGTACCGCAGAGGGGGCAGGGGTCTGAGCAGGGACAGGG  
 TGGGCGCGCAGGAAGCCCCCTTCTCTGTGAGGTGCCCCGGCCCTGGAGCCTCTCTGGG  
 GCATGCCACCCCTCTCACAGACGCTCCAGGAGCCCCACTTTCTGCTGCGTGGTGAG  
 GGTGTCTCTACCCGATTCTTGGCCCTGCAGGTGAGTGTGAGTCCCTGCTAAGCCTGGGG  
 TTGGAGCAGGTGCAGGGCATCACACACAGCAGCAGAGGCTGTGGGGGCCCTGAGAGGC  
 GCTCCAGGTACCTCTCAGGGGGTGAAGCCGGGGTGAAGCCGGGACCTCGCCTGCCCC  
 CAAAGCCGGCGCCCTCTCCCGCCCCCGACAGGGCCAGAGAAGCAGGTGTGGGGCGG  
 CACAAACCAAGTCACTTCCAGATCCTGCTGGGGCCCGTTGAAACTCGAAGCCCCCAG  
 GCTGGGAGGTAGACACCCCTGCCAGACCGACAGCCTGGGCCTGGCTCACAGCTGCCT  
 GGGGGCCAGGGGTGCACCTGCCCTGTGGGTGGGGTCAAGGGCAGGGAACCCCTCGGGA  
 AGGTCCCCCAGGGTCAAGGTGGGGCTAAGCTCCGGTGACCTCTGGGAAGTCTGGGGCTG  
 GGTTTTGTTCAGAGGAGAGAGGGCCAGTAGCCTCAGAGGGGCTGTGGCACGGTGGGAA  
 GGCCCCAGGTGACCCAGAGCGTGCAGCAAGCCCCCTTGACTGCAAGC  
 GCAAAGGGCAGAGGTGGGGTGGGAGCCTCGACCCCCGAGCCAGGTACACAGGGGGAAG  
 GCGAGGGATCCGCGAGGGGCCACACCCGCCACCCAGGCAGCCACAAAGCCTTTGGGC  
 CCGGAGCCCCAGATGGGGCCAGCCAGCTCTGGGAACAGTCTTCCAGAAATTTCCAGCT  
 CTGGGTACCAACAGGGCTGCCCGCCCCAGAGCCCTCGGGCGGGAGACCTTCCCCAGG  
 GGGATCTCCTAAGTGGCAAGGCCTGTGGGAGGGGCTGGTGAGAGGCCACTCTGGCGGGA  
 AGACCCCCAGCCACCTGGAGCCCTAGCCACTGCCTGCTGCGGCTCCCTAGGGATCCAGG  
 GCCATCAGAGAAGCTCCAGCGACACTGTTTATTTTCAAATGACACTTTTAAAGAAAAACA  
 GCCTCACCCAAATGCTTGGCCCTGAGTCTGGAATGTGCAGACAGACAGCTGCCCCCTCCC  
 AGAGCCTGCACGGCCCTCCGGGTGGGGGAGGAGCAGGGGGCACCCCTGGGACCGGGCCG  
 AGGCTGTACAGGGCACGGAACGTGTCTGTGGCCCTGTCTCAATTTCCCGGTGCCAGTGG  
 CCCCACCTTCCAGCAGACCCAGCAGGGCCCCAGCTTGTCTTGGCTGGCCGCTGGTCTCT  
 GTCACCCAGGCCTGGAGTTCTGGAAGATTCTGCTCCTGCTCCCGTGTGCACATACCACT  
 CCCCAGGGGAGCCCTGCACTTCTGTTCTGCTGGGCTCCCTGCTGATCCGTGAGGCCCT  
 GCAGCCCGCTGATCTTCCAGGTCTCTCCAGAGCCCCCGCTCCAGGAAGCCCTCCAGG  
 AGAGCTCAGGAGGGTGGGCTCCCTGCGCGCAGCTGTGACACCCCTGGGGCCACCCCGCG  
 GCTGCTAGGGTCCAGGTTCCCCACAAGCCCTCGGGCAGAGGCTGGGCGCTGGGTCCCTC  
 GGAGACAATGGCTCCGAGGCCCTGCCCTAGACGGGTTTCCGGGAGCCCGTCCCCAGCGG



FIGURE 8, CONTD.

CACCCACTGAGTTTTGAACACTTGGCGCCACCCCCACACCCAGGCGGTGGCCAGGAGGC  
 CTCCTGGGCAGCAGACAGTCCGTGAGGTGGCCCTGGGGTGGCTCCTGACCTGGGCGCTGG  
 CCCAGCCCTGGGCACAGCTTTCCAGATCTTGCCCTGCCGCTTCTCCAGGCTGCCTCGGCC  
 CCTCCCGCTGGGGGTGCCAGCTTTTCTGGAGGATGCCACCCCTTGCCCATGGTCAGG  
 GAGGGGTGAGAAACCCACCTCGTGCCTCTGCCCGGCTATGCCAGGGGAACAGGTTTC  
 CCTCCCGCAGGAGGGGACCGAGTCCCTGACAGCCCACTGCAGAGGGGAGGAGGTGCCTGG  
 CTCTGCCCCAGCCCCACCAACCCCGTGGCTTCTGTTTCGCAGCCCAAAAGCACTAAA  
 GGCCGCAGGTCTTGAACATCAAAGACCCGGAAGTCCATTGTATTGAATTGAGTGTAAA  
 TGAGCCTGAGGCCTGTGGCTTGCCTTTCCACAATTACCGCTGCCCGGAAGGGCTCCGG  
 AACCGACACAGCCCCAGGGCCCTTGCCCATGTGGGGAGCCAGGCTGGCCTGAAGAAG  
 CCCCATAAGGTGGACCCCACTTTGAGCCCCACGAGAGTGGGCCAAGGACCAGGTACAGG  
 GCTGCCAGGCTCTGGGCTCCTCTGCCTGCCAGGTGGGCTCCCTCGGGGCCAGCCTGG  
 CCTGCAGGACCTTCCCACGTGAGTTCCTCCAGCCTGGTATGAGCGTAGTGGACGGCAGCC  
 ATGCCCAGCACTCAGGGCCTGAGGGACAGAGCGGGAAGTCCAGCCCCGGGTCTCTCGGC  
 CCTAGGATCCTTCTAGGTGGGAAGCCCAAGGAGCAGAGGGGTGAACGCAGCTGTGTG  
 GGGCCCCAGGCTGCCGAGCAGACCCCTCTGCTCCACTCCTCGGCCGAGTGGGCGCCGAG  
 ATGCCGGGGCAGTGCCATTTCCAGGCGCCACCGGAGGCTCCCAGAGGGAGTGAGGCACG  
 AGCTGGGAGGGAGGGCGGGGGGCTGGGGAGGCAGAGAGCGGAGGCGGAGGCCGGTGAG  
 GAGGCCCGGAGGGGGCTGGAGTCAATGACCCAGGGATTATCGTGCTGGGTCTTTGCAAA  
 GTTGGCTGAGCAAACGCGCGGAGCCCAAGGGTCAGGGAGACGGGACTGGCGGGGGCCCGGG  
 CCCCCCTTTCCCTTTCTGAAAAAGCCTGTTTCCAGGTCAAATCCAGCTCATGATCCG  
 CCCCCCTTTGGGACTGATGTTCAAGGCCCCAGTGGTCCCAGCACCTCTGTCCACCGCCCC  
 CCCACGCTCCCGGGGGCCGCAACCCCTGTGGGCTGCGAGGTGCGGGCACCTCTCCCTTCG  
 AAGCAAAGCCCTGCCCTGCGTGGGCAGCGTGATTTCCTGCTTCTTGGGGCTGCACCTTG  
 ACTGGGGTGGGGGGGTGG

**Contig 4 (1592 bp)**

AGCCCCCTCAGCCCCCTCCGAGCAGCTGCTGGGCTCAGCGGGCTCGCCCCCGATGTGCGGC  
 CCTCCATAATCAATCATGAGGGCCGGGCCCGGGGGGGGGCGGGCCGACCTGTAGCCAGC  
 TCCAAGGGCAGGGACAGCTGCTGTTCCGGAGGGTTCAGGGGGCCAGCCCCACAGACAG  
 CGGCCCTCGGCCCCCTTCCCGAGGGGCACCCCCACGGAGGGCCAGACCGGAGGGACTC  
 GGGGCCCAGAGGCCAGGGCAAGAGTGAAGGCAGCGCCGGTGGGAGCGGCGGTGAGCGGG  
 TCCAGGCTTCAGTTCCAAGGAGCCCCATGCCCTGAGCCCGCACTGAGCCCTGTGCAGCC  
 TGTGGGTGCCGCGGAGGCCCCGCCACCCGCCCCCACCAGCCTGGGGTCAAGGAGGGAG  
 GGGGTGGCCTGACGGATGGTAACAGCTGCTCCCCCACCTCGCCGGCGTGGACAGGGCTC  
 GCCTTCTCCTGCCGAGCCCCCGGCTGCCCCATCCGTACGGCCCCACCCAGGACTGTGCGT  
 CCAGCCTCCCTCCCTCCTAATCCCCCGCATTTTCCGAATTCTCGGGCACTGCTGCTTC  
 CTCTCAAATTCCTGGCCCCCTCGCCCCATCCCCGCCATGGGAAAGGGCCGCGATGCCA  
 GGACACTTGCTCGTCTCGGCCGGGCGGGGGAGGAGCAGCTGGCTGGGCCCGGCAGCTGT  
 GAGGTGCGGGGGTGCCAGGGAGAAGGGCCAGATTAGGGGGCGTCATGGGAAAGCTGGGA  
 GGAACGCTACCCAGAGCCCCCTGCGCGCAGCCTGTGCTGCTCCCTCTCCGCATTTCTG  
 GCCTCTGAGTGTCTCCCTGGAGGGAAGGGACCCTGTGCTGCTGCTGCTGCTGCTGCTGCT  
 AGGAATGTCCATCTGTCCGGGCGGGTTACCTGGCTCAGAGCGTGGGTACCACTCATCC  
 AGCCCTGACGCTGCTCTCGGAACAGTGGATGGGCCAGGCGCCCCCGTCACACCCCGCA  
 GCTGGGCTCCACAGACGGGCGCGGATGGCCACGAGGTGGGGGGCGGGCCAGGGCGAG  
 GCTCCCTCCTGGAAGGGCTAGAGTGTGGGCTGCGCGGAGAGGGAGGCGGACGGCCAGGC  
 CAGGTGCAGCCCGGGGAGGTGCTGGTGGGGGCTGTGACCCACGTGTGCAGCTCAAGGGT  
 CCAGGAGCCCCAGGGACAGAGCCTCAGGGACAGACCCCTCAGAGCCACAGCAGGAAGCCTG  
 GTGGCAGTAGCTGGCGGGGCGGTGGGGTGTGCGCCCTGCAGACAGAGGCAGAGGCAGGC  
 TCCCTGCTGATGACAGGGGCTTTCTCTGTCCCTGGGGGGCGGAGGGGGCCCGACCATGG  
 ACCCGGGGCTCCTCTCGCACGATTCCAGGCCAGCCTGGTCTCAGGCAGTCCAAGGTTG  
 CACAATGGTCTCCATCGTCCAGAGTTGCAGAGCCAGCACTCTCCCACTGGACGGCGGGCC  
 GGGGTGGGCTGCACCGCCGCTCAGGGCTCAGGGCCGCGGCGCCAGCCNCCGAGGCC  
 TTGACCTGTCTCTTATACACATCTCAACCCTG

**Contig 5 (831 bp)**

TGAGATGTGTATAAGAGACAGGCCTTGACCCTGGGCTGGCTCAGCTGCGCGCCCTCCTC  
 CTTGCAGCTCCGCTCGACCCATCCATCAGCCATTTTCTACCTTCTGTAAATAAAAA  
 ACCCGAAGCGGCGTGGCCCGGTGTCCGCTGGGGTGAAGTGCAGGCTGCTGCTGGTGGCTC  
 CCACCTGGGCCCCGGCCCCCTGAAACACACACCCGGCGATGGCTTGGCCGGGGCCCTGGT  
 GGAGGGGCGGGGGGCTCGCTGCTCTGTGTAATTTTCGGTCCACATGCCCCGAC  
 TCCTCTCCCGGCCACCTGCAGGCCCGGCGGTGCCCCGGCACTTTCCCGAAGGACGG

44/48

FIGURE 8, CONTD.

ACTCAGCATTTCCAGGGACCTGCTGATGGTGCCAGACCCCGGGGGCCTTCCCGCCGG  
GCGCGGGCCCCACGTGCCCCCTCCAGTGGCCACAGCGGGCCTGGGCCAAGGCTGGGAGTTC  
TGACCGGGCCTGGGGGAGGAAGGCGGGGAGAGGGGACACTCTCCTGGCGGGGACGAGGG  
TGGGGGCGAGCAGGTGGGGAGTTCCACAGCCGGGGCAGCGGGACGCCGCTTGGCTGCCCT  
GGGTCTCAGCCGGGGACAGTGGCCACCAGGAGAGAGACGGCAGACAGTACAGCCCACCCG  
TTTTATATCCTCTCAGGCGGTCTGTGCTTTATTGGGGTAAATATGCAGGACATAGAACT  
CTGCCACTGGACCCCTTGGCCGGGGGACACAGCAGCGGCATTGCATGCTTCTGGGTGCA  
GCGCAGCCAGCACCACCGGCCAGAGCACCCCATCTTCCCGATCAACCGGAC

## Contig 6 (4634 bp)

CTCTGGGCTAGCACCGTGGGGGCTTTGCCAGAGTGGAACTGAACTGGGTCCACCCCGGAG  
CCCAGAGGGCGGTGAATGGGAGGCAGAGCCCATCCTGGGAATGGACCAGAAGAAAGGGAG  
CGGGGGTGGGGGAAGGGGCATCAGATCCTGGTCTTCTTGTGCGCTGCGGTCCCTCTGC  
CACCCTCCCCGAAGCTGATCTGGAGCACACGCGTCGTTAAAGCCGCCATCGAGGGCCCA  
CTTCTGACAGACGGAAGGGGGCAGAGTGCCTTCCCTACCGGCCTCGCCCTGGGAAGGCCC  
CTCCCTGCAGCCAGGAAGCCAGCAGAGGTGACAGAGCCAGGGGCCAGGGCCCCAGGG  
ACGGGCTCGCGCGCCCGAGCCGGGGTCCCTTGGCGTCCCCATCCTCTCGTCTGGAGCC  
CTCCTGGGTGACCACAGGAATGTGCAAGGCGGCAGCCGGGTGGCGGCCGGGAGGCGGGTG  
GGAGGCGGGGCGGGTGGCCTCTTACGGGCGGGCCTGAGAGATGGGCGCCCGTCCGGCCC  
TGGCGTCATCGTCTCCGCGTCTCTACCCACTGAGCAAAGACACACGAAATGAAGCTCGAA  
CGAGCACAGCCAAAGAACGGCCGTTTCTGTCTTTCTTCTTAATCCCTTTGGCTTAGGGT  
TTCCCGGCTGGACAGCCTGCCCAAGGCGACATGGGCATCCGTCCGGGGACATTACGGCA  
GTGACCAATCCCAGGCCACCCAGGCTGTGCCCTGCGTCTGGGCCATTGCCAGCCGGCC  
AGAGATGGAGCAGCCACTCGGGTCCCCGAGTCTCGGTGAGACAGTCAAGGATGGACCTT  
GGATGGAGACCGGCGTGCGCCATGTCCGTGGGTGAAGGAGGCGTGCAGGCCGTGTGGG  
GGACATGGTTGCTGTCCCTCGGCCAAACCATGAAAGCAGCCCTCTCCCCCAACCCCCA  
GCACCAACCCGGAGACCACTCGGCCGGAGCCAGCAGCGCCACCGTCACGTCTCGGT  
GTCCAGCTTGGGACAGGTGAGTTCCAGATGTCCAGGCTGGAGCTGGTCTTGAAGATCC  
TAGGGGTCCAGCCAGCAGAGGGGCCAGGTGAGAGCCCCCTGTGGTCTAAGGATGCA  
ACCAGGGGCCGGCGGGGTGCCTGCCCTAGAGGGGGTAACTCGGCCCTTGGGGACAGTC  
ACCCAGGAGGTCCCCAGAGCCAGCTCGGAGGGCCACAGGTGCCAGAGTCCCACCTGG  
GGAAGGCTGCCCTCTTCCAGCCCCGAGCCGGGCCCTTGGCGCCCGCGTCCAGCCGG  
ACCCCGGGGAGATATTACCCCCCTGCCCGCTGAATCAGGAGGCCCCGAGCCCATGTTTT  
CAGTCTTTTCTCTCCATCCAGCCCCCAGGAGAAGAGGTGCTGAACTGGGTCCCCTGG  
AGGCTCTGAGCCCCAGAAGTGCCTCTGAGCAGACGGGCACTCTCAGACCAGCTCAC  
GCTGGACAAGTCAGTCTCTGCTGCCGCTGATGGGCCCTTGGGAGAAGCAGACATGGTG  
AGGAAAAGGCCCTGTGCCCTTACCCCTAATTTCCCAAGCCCCAAGTCCCAGTGGGTGCC  
AGCTTCAACCTAAGCAAATAATTCGTGCCCTCTAAACAAACGCGGGGAATCCACCTGC  
CCTTCCCCCGCCCCCCCCC  
ACCCCTGGCCTTGACCTCCAAAAGCACTTGAGGGGGCTTTCTCCAGACACCCTCCAACCC  
CGACCCCATGAAGAAGGGGTGATGGGCTGTTACCCCAACAAGCAAGAGAACGAAGCCCA  
GAGAGGAGTTGGCGTGGACAGCAGGGGTGAGGCCCTTTGCCCGAGGGCAGGGCTGGTG  
CCACCTGGGTGAGGCGCAGGCCCTGGAAGAGCACCGGAAATGAGCACACCTGGGTCTCT  
AGAAGGTTCTTCCAGACTCTGGGGGTGAGTCAATTTCAACACTCCTGGGCCGGCAGGG  
CTTCTTCTTGGCCCCGAGGGACAAGGTCCCCTTCGTCCGGGGGTACGGCCCCCTGGACCC  
CTGTCCCCCGCACCCACCTCCGCTGGTGAGGGCCGCGGCAGCTCTGGACACAGATC  
CCTCAGAGCCCCTTCTCCCTCCCTGCTCCCTCGTCTTCCCAAGATGCCCGGCCCTCCAGG  
TGGGGCAGCCAGGCGGCAGAATGTGGTCCAGGCCTCTCGGCCCCACCCACACCCCCCTGC  
TCTGCCCTGACAGCCTCAAGACGAGGCACGTGCTGCGTCTGCGTCTGTCTCTCTCA  
TGGCACAAAACGGTGCCCGCCTAGCTTCCCCCAGAGAAGGGAGATCGTGCTCCCCGGACG  
GACCCCTGCTCTGCTGTCTTCCCGCCCGGCTTACGGGCTCTCCCAAGGGTGGCCGCG  
AGGAGGCCCTCGCCTCCGGCCACGGGGCTCCATCCTCCGAGCCCGACAGGCCTCCGCG  
TGGTGGTCCGACCTCTTCCCAAGGCCCGCCATCCTCCTCGCGCTCCCCCAAACCTG  
CCTCTTTCCCCAGCGCCCTTGTCCCCACGGAAGACCTCCACCCGTGCCATTACACGCTC  
TCGCCCCACCTCCAGCCACCCCCCTTCCCCATCCTCCTGGAAGCTCCCACTTCCTTC  
CCGTCTCCACGCGCAGAGAGGGTCAGAGCTCAGGGGTCTTGGGGCGTGGAGATGGCC  
TGCCCCGGGGTCTCGCTGACCGCCTCTACGGAAGCTGTGCCGGGGGTGGGGGTGTCTC  
TGCCCCAAGCGCTGGAGGACGAGCCACATCCAGGGCAGCCGGAACCTGCGTCTGGTCT  
GAGACGGAGAGGCTGGGTGACAGTGGGTGAGGGGCTGCACACAGCTTGGCTGGGGTCC  
CCTAGGTGACAACACTGGCTGAACACTCATTTGCTGCTCCCTTCCAGGGTGACCTGGGG  
TCCCCGTGTGGCCCTCAGGGCACAGGGGGCCCCACAGGCCTCACAGAACCCTAGTGGG  
ACTGCACCCAGGGCCACAGAAGTGGGGGGCACTGGGGGTCCAGAAACAACCCCAAC

FIGURE 8, CONTD.

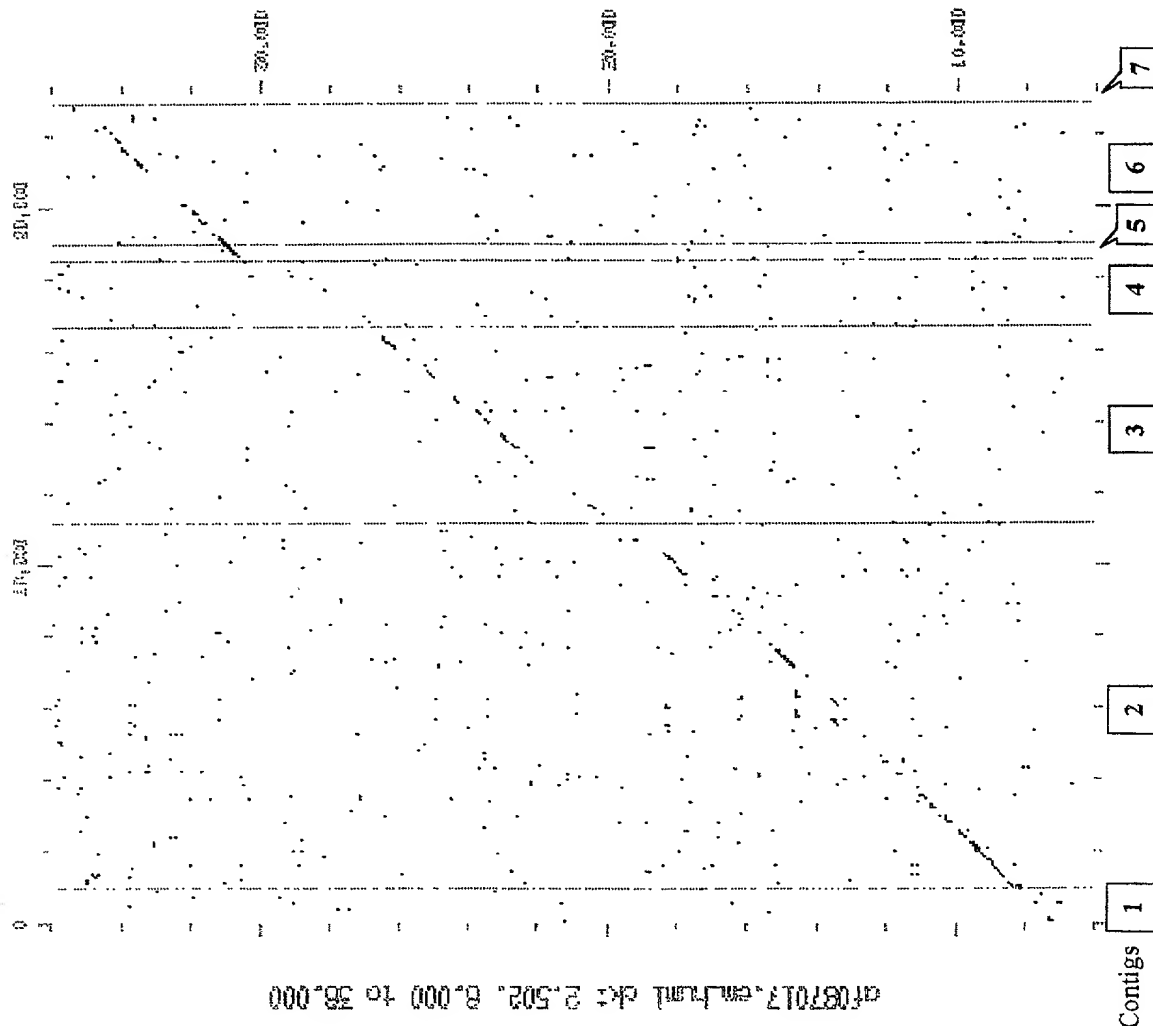
CAGGCCAAGGTGGCCAAGGCCTTACTCGAGCGGGGCTGCCCGTCCCAAGAGACTCTGGCC  
AGTCGTCCGGATCCAGCTTCCCGGGGCCGGGCCCGCTGGGCTCCAGGCGGTTCTGGG  
GGGCCCTCCCCGGGGGTTCCGCTCTCAGCAGCAGGAAGAGGAGCGCGGCCAGC  
GGATGGGGAGAAGAGGGCGCCCTGGCCATCTTGCTCCCCCTGGGACTTGAGGAGGGTCTC  
GGGCCGGGACAGCGGGACCGGGAGCCACAGAGACCCTGGAGGAGGCAGCATGGCGGGGAG  
GTGACCGGGGAAGAGGGCCGTGTCCAGGCTCACAGCCCGGCTGGCCGCCCGGCCCTCG  
GGAGGCGTGCCGCTGACCGCTGGCCGGGAGGTTTGCTGCGTGTGGGGTTTGCAAAAGT  
GCTGAGCTGCTGAGCCACAGGCCAGGCTCAGAGGGGACAGGAAGGAGGTTGCTGCCAG  
CCTCGGGCACTGCTGACCCATCTCCGTTTCCAGGGCACCAGAGCCACCTAATCTGCCGG  
CTCTGTGCCAGGGACAGGCTTGCTGATCTCTCAAGGCCGGGCGCTCCGCTTCCCTGG  
GAGAGGGTTAAACATCCAGCCCCAGCCAGCATCTCGGGCAGGTTCTTGCTCCCCCGCT  
CGTGCTCTCTGAGACCCTGGTCGGCACACCTTTCCTTGAGAGGAGGAGGAGGAGGAA  
AGCGGATGGAAACCAAGTGACCCCTGCAGCCCTGAGGGCACCTTCCACGTGCCCCGCCCC  
CCCCGCTCCTCCGCCCCCAGTTCTCACGGCCCCAGTCTGATGGAGGGAGGGCGACCTC  
CGGGCTCCTTGCTCCCGCCGGCTCCGGAAGACAGGGCCGCTCGGCTGGGGCTGCAGGGA  
GGGGCCCGAGACGCAGGAGAGCAGCCCGGAGGCAAAACCCGCGGGTCTTCCAGAAGGAGG  
CCTGGCAGGGGGAGGGGGGTGCCACCACTGCTGTCCTCTCGTGCCACAGTGGAGGGTGT  
GGGTGGGCAGTGCCGGGTGGGAAGTGCAGAAAGACCCTGGACCGTGGGGCTGGGGCCGCC  
ACGGGGGAGCGGGGTCTGTACGGGACCCCTGGGGGAGGGAGGCGAAGGGCTGGGGCAGAGG  
CCGGATCACTTCCAGATTTGCTGTGGGACCAAGGGCCGGACCTCGGGGTGACTTCTTTTG  
TGTGCTGGCCACAGGGGGGCCCCGGCGAGGTCACACGGAAGGGGGCTTCGGACCTGGCCT  
AACAAGCCCACTCCCGAGGAAGATGCAAGGGGAGGCAGACGGAAGGGCCGAAGGGGGCGA  
TCGGGGGACACCGCGCAGGGCCGGGGCAGAGAAGGGAGGCAGAGGGCAGAGAAGGGAGG  
CAGAGGGCAGAGAAGGGAGGCAGAGGGGCCACATGCTTGGAGGGCCAGGGAGGAGCGGGA  
ACGGCGTCCGGCGTCCAGCGCCGAATCAGGCCCGTACGGCGGAGGGTGCCTGGACCTGCC  
TGGCCTTCAGGACACAGTCAGCAGGCTGTCTTTATACACATCTCAACCATCAT

## Contig 7 (482 bp)

AGCAATGGGGCCGTGACCTAAGGAGGCAGGCCAGGTCAGTGGGGTGACCTCTCGTGGCC  
CCGATGTTTGGAAATCCCCAAATCAAAATGACCCATCCGACAAGCTTGATGCCTGCAGG  
TCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGCCCTATAGTGAGTCGTATTAC  
AATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCTGGCGTTACCCAACTT  
AATCGCCTTGACAGCATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCCGACC  
GATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCCTGATGCGGTATTTT  
CTCCTTACGCATCTGTGCGGTATTTACACCGCATATGGTGCACTCTCAGTACAATCTGC  
TCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGAACCCC  
TT

FIGURE 9

Human clone af087017.em\_hum1: H19 gene + flanking sequences



DOTPLOT of: seq24kb.pnt Identity: 34094.32 December 6, 1998 12:40  
 COMPARE Window: 21 Stringency: 17 Points: 3,497

FIGURE 10

IDENTIFIED POLYMORPHISMS:POLYMORPHISMS TYROSINE HYDROXYLASE GENE - CONTIG C3 (figure 6)

1	GGATCCAGCC (A:T) GCAGCC	1081 bp
2	ACAACCCCC (-:C) TCCCACAG	1149 bp
3	TGCGGAGGGG (A:G) GACCTG	1186 bp
4	AGGT (CAAGGCCAGGT: -) CGAGG	1210 bp

POLYMORPHISMS INSULIN-IGF2 - CONTIG C4 (figure 6)

5	CCC (C:A) CCCC (A:C) CGCCGC	438 bp
6	CCC (C:A) CCCC (A:C) CGCCGC	443 bp
7	CGCCGCAGCA (G:A) GCCG	455 bp
8	GCTTATGG (G:A) GCCGGG	503 bp
9	CACGGC (T:C) TC (G:A) GAGCA	525 bp
10	CACGGC (T:C) TC (G:A) GAGCA	528 bp
11	GTCTGC (A:G) GGCAGGTG	571 bp
12	CAAGCCCGG (G:T) CGGTT	636 bp
13	ACCTC (A:G) AGGCCCCCA	710 bp
14	GC (C:T) GGGCCCAGCCGC	867 bp
15	ACCAGCTG (C:T) GTTCCC	903 bp
16	GGC (C:G) CTCTGGGCGCC	1148 bp
17	GGGGG (C:T) GTCCCGGGA	1305 bp

FIGURE 10, CONTD.

18	GCGGT (C:T) GGGGGAGTT	1320 bp
19	CGCCC (C:T) GGTCCCGCT	1400 bp
20	TCCC (G:A) TCTGCCGGCC	1519 bp
21	GA (T:A) GCCCCATCCCCC	1547 bp
22	GG (C:T) GGCTGCTGCGGC	1607 bp
23	TGGCTGC (G:A) GTCTGGG	2222 bp

POLYMORPHISMES IN CODING REGION - CONTIG c10 (figure 6)

24	GCGCA (G:T) TGATTGGCA	341 bp
25	CGCCCCCCCCC (-:C) (G:C) GG	2247 bp
26	CGCCCCCCCCC (-:C) (G:C) GG	2248 bp
27	GCAGCCGGCTC (C:T) TGG	2257 bp
28	GTTGTTG (C:T) TCTGGGA	2413 bp

MICROSATELLITES

29	PIGQTL1: (AT) <sup>11</sup>	112 to 133 bp Contig 57
30	PIGQTL2: (GT) <sup>8</sup> GCACGCGTGTGCGTGTGTAC (GT) <sup>17</sup>	1074 to 1144 bp Contig 95
31	PIGQTL3: (CA) <sup>19</sup>	223 to 260 bp Contig 105

**Declaration and Power of Attorney Patent Application  
(Design or Utility)**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: 'Selecting animals for parentally imprinted traits'

the specification of which

- ☐ is attached hereto  
x was filed on June 15, 2001 as application serial no. 09/868,732  
and or PCT International Application number PCT/EP99/10209 and was amended  
on (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information know to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or 35 U.S.C. §365(b) of any foreign application(s) for patent or inventor's certificate, or 35 U.S.C. §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate of PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)		
Number 98204291.3	Country EP	Day/Month/Year Filed 16-12-1998
Number	Country	Day/Month/Year Filed
Number	Country	Day/Month/Year Filed

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

Prior Provisional Application(s)	
Serial Number	Day/Month/Year Filing Date
Serial Number	Day/Month/Year Filing Date
Serial Number	Day/Month/Year Filing Date

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s), or under 35 U.S.C. §365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

Prior U.S. or International Application(s)		
Serial Number	Day/Month/Year Filed	Status (patented, pending, abandoned)
Serial Number	Day/Month/Year Filed	Status (patented, pending, abandoned)
Serial Number	Day/Month/Year Filed	Status (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



## Power of Attorney

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Attorney	Registration Number
David V. Trask	<u>22,012</u>
Laurence B. Bond	<u>30,549</u>
Allen C. Turner	<u>33,041</u>
Edgar R. Cataxinos	<u>39,931</u>
Samuel E. Webb	<u>P44,394</u>
William S. Britt	<u>20,969</u>
Joseph A. Walkowski	<u>28,765</u>
Kent S. Burningham	<u>30,453</u>
Brick G. Power	<u>38,581</u>
Eleanor V. Goodall	<u>35,162</u>
Thomas J. Rossa	<u>26,799</u>
James R. Duzan	<u>28,393</u>
Robert G. Winkle	<u>37,474</u>
Kenneth C. Booth	<u>42,342</u>

(14)

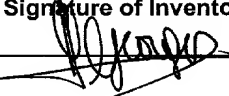
I hereby authorize them or others whom they may appoint to act and rely on instructions from and communicate directly with the person/organization who/which first sends this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instructed otherwise.

Please direct all correspondence in this case to at the address indicated below:

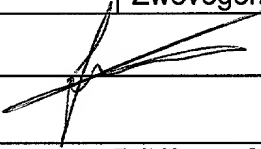
TRASK, BRITT, & ROSSA  
P.O. Box 2550  
Salt Lake City, Utah 84110

Full Name of Sole or First Inventor		
Family Name Andersson	First Given Name Leif	Second Given Name
Residence and Citizenship		
City of Residence Uppsala	State or Country of Residence Sweden	Country of Citizenship Sweden <i>SEX</i>
Post Office Address		
Street Address Bergagatan 30	City Uppsala	State & Zip Code or Country S-752 39
Signature of Inventor <i>[Signature]</i>		Date July, 21, 2001

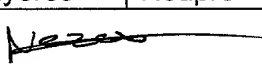
2-00

Full Name of Second Inventor, if any		
Family Name <u>Georges</u>	First Given Name <u>Michel</u>	Second Given Name
Residence and Citizenship		
City of Residence <u>Villers-aux-Tours</u>	State or Country of Residence <u>Belgium</u>	Country of Citizenship <u>Belgium</u> <u>BEX</u>
Post Office Address		
Street Address <u>Rue Vieux Tige 24</u>	City <u>Villers-aux-Tours</u>	State & Zip Code or Country <u>B-3161</u>
Signature of Inventor 		Date <u>13-8-2001</u>

3-00

Full Name of Third Inventor, if any		
Family Name <u>Spincemaille</u>	First Given Name <u>Geert</u>	Second Given Name
Residence and Citizenship		
City of Residence <u>Zwevegem</u>	State or Country of Residence <u>Belgium</u>	Country of Citizenship <u>Belgium</u> <u>BEX</u>
Post Office Address		
Street Address <u>Sint Denijsstraat 26</u>	City <u>Zwevegem</u>	State & Zip Code or Country <u>B-8550</u>
Signature of Inventor 		Date <u>13-8-2001</u>

4-00

Full Name of Fourth Inventor, if any		
Family Name <u>Nezer</u>	First Given Name <u>Carina</u>	Second Given Name <u>Danielle. A.</u>
Residence and Citizenship		
City of Residence <u>Neupre</u>	State or Country of Residence <u>Belgium</u>	Country of Citizenship <u>Belgium</u> <u>BEX</u>
Post Office Address		
Street Address <u>7, Impasse des Bruyères</u>	City <u>Neupre</u>	State & Zip Code or Country <u>4120</u>
Signature of Inventor 		Date <u>13-8-2001</u>

# SEQUENCE LISTING

<110> Andersson, Leif  
 Georges, Michel  
 Spincemaille, Geert  
 Nezer, Carine

<120> SELECTING ANIMALS FOR PARENTALLY IMPRINTED TRAITS

<130> 2183-4951US

<140> US 09/868,732

<141> 2001-06-15

<150> PCT/EP99/10209

<151> 1999-12-16

<150> EP98204291.3

<151> 1998-12-16

<160> 123

<170> PatentIn version 3.1

<210> 1

<211> 21

<212> DNA

<213> Pig

<400> 1

ggcaagttct tccgctaata a

21

<210> 2

<211> 20

<212> DNA

<213> Pig

<400> 2

gcaccgcaga attacgacaa

20

<210> 3

<211> 26

<212> DNA

<213> Pig

<400> 3

gtttctcctg tacccacacg catccc

26

<210> 4

<211> 19

<212> DNA

<213> Pig

<400> 4

ctacaagctg ggctcaggg

19

<210> 5

<211> 25

<212> DNA

<213> Pig

<400> 5

cccctgaact tgaggacgag cagcc

25

<210> 6

<211> 25

<212> DNA

<213> Pig

<400> 6

atcgctgtgg gctgggtggg ctgcc

25

<210> 7

<211> 19

<212> DNA

<213> Pig

<400> 7

cgccccagct gccccccag

19

<210> 8

<211> 19

<212> DNA

<213> Pig

<400> 8

cgccccagct gccccccaa

19

<210> 9

<211> 20

<212> DNA

<213> Pig

<400> 9

cctgagctgc agcaggccag

20

<210> 10

<211> 500  
 <212> DNA  
 <213> Pig

<400> 10  
 ggggtgggcag cttcctccca gaccgcagga ggcccaagtt ccttggccct gccacccag 60  
 ggccagctga agcaggtcag agacacccgc tctgtccct cctgtcacct aacccaacag 120  
 gccggggccc agggacacag gccacatggc atctccccc atgccctgc cccaaggcgc 180  
 ccagcaggtg aggctggagc agagtctggg tcttgcgggc cagaccgagg gcaggacagc 240  
 tgggcatctg tcctcacagt ccccgcgctt tgtcgggagg cggcagagcc tcatccaaga 300  
 cgcccgcaag gaacgggaga aggcggaggc cgcggtgcc gcgtccgagc ccggggaggc 360  
 cctggaagtg ggggcccttg ccgagcggga cgggaaggcc ctgctgaacc tgctcttcac 420  
 cctgagggcc accaagcccc cctcgctgtt ccggtccctg aaaaaattct aggtgagggg 480  
 gcggggccagg gctccccggg 500

<210> 11  
 <211> 943  
 <212> DNA  
 <213> Pig

<400> 11  
 tgctcctcac accccgggag gggctgtctt tggggccatc ctccccatgg gccagcacc 60  
 cactctggcc ttcacacctg ccgtcttctg ggaagtcctc tggttcccaa ggaaagtctc 120  
 tgagctggac aagtgcacc acctggtcac caagtctgat cctgagctgg acctggacca 180  
 cccggtgagc cgggtgcctcc cctccccggc cgccatgtct cccatcccca ggggtgtccc 240  
 cacactcagg gccgggactg ggcgtgaacc ccgggttggg acggatgttg gcctgctgtg 300  
 tggctcctgg cggaacagag aggcctggct gggtgccacc ccagggccc ccgccatga 360  
 cacgggccgc gtctgggctg ggcgggcagg gcggccaggc agggcagcct ccgatggcgt 420  
 ccccggtgtg caccagggct tctcggaaca gttgtaccgc cagcgcagga agctgattgc 480  
 ccagatcgcc ttccagtaca ggcagtaagt cctccaggg cctcagcctg ggggcccaga 540  
 cctcagcctg ggcctcacgc cagacctggg ggtggaggga agggagggtg tctttgtcac 600  
 caacgccacc accttcaactg tcaccatggt caccgactct ggggtcccaa atcacagctg 660  
 aggaaactgg ggcacagagt ggttaagcat cttgctgaag ccacacagct ggcggagcat 720

ttggccccgg cccctcctgc ggctcccaca cgtgctccct gaggggcccc ggactgacag	780
ctgtcccctc ctgagagggtg accctattcc ccgcgtggag tacacagccg aggagattgc	840
cacctggtga ggccctgtga cagcggctgg gaggggcccc agtgggggaa gggacaggaa	900
gacctcagaa ttcccgctg gaacgtggtg gcctctatca tga	943

<210> 12  
 <211> 1500  
 <212> DNA  
 <213> Pig

<220>  
 <221> variation  
 <222> (1081)..(1081)  
 <223> Polymorphism A:T

<220>  
 <221> variation  
 <222> (1210)..(1210)  
 <223> Polymorphism CAAGGCCAGGT:-

<220>  
 <221> variation  
 <222> (1186)..(1186)  
 <223> Polymorphism A:G

<220>  
 <221> variation  
 <222> (1149)..(1149)  
 <223> Polymorphism -:C

<400> 12	
ggggagggga tgctcagacc cgctctggga agaagagagc ctcagaagaa atcccttccc	60
aagggtcacg cgggtggagcc caggggcccc ctaggggccg attcccacag ctctgtctgc	120
cacctgctgg cgctcccagg aactgcggag gcgggtgggg ccctggatgg gtccggcagt	180
gggctcgag gagacccctg gaggggctgc ggacacccca gctgccactc acaagggtgcc	240
caagcggcgg tggcaatggg ctgagcctct cccccctcc tcctccgcag gacattggcc	300
tcgcatccct ggggggtctcg gacgaggaaa ttgagaagct gtccacgggt ggtttctccc	360
cctgcagggc cctgggttcc agccaggccc tcctgtccaa ggggtgtcgt cctcacgctg	420

tgaccgcccc	ggagcctgga	tcggttctgc	ctgggtgggc	ggtgccccgg	ccacgggcag	480
caggggcagc	ggtgcccccc	ccagccgtgt	ctgagcccc	ttgccgcctg	tccccaccag	540
ctgtactggt	tcacggtgga	gtttgggctc	tgcaaacaga	acggcgaggt	gaaggcctac	600
ggggctgggc	tgctgtcctc	ctacggggag	ctcctggtga	ggcctcccc	acgcgctggg	660
gcctgggtcc	ccgggggagg	tgacccctgc	ggtgccttgt	ggattccagc	tctcgggagg	720
ctggagcgag	gggctgccct	cctgggggca	ccaagaaagc	tggctctgcg	ccctctccac	780
acacctgtgc	ctgggccctg	gggggacccc	tgctggggga	tgtgggtgca	cagccagggc	840
caccagggag	tcaggacacg	gggctccctt	ccctcgggtc	cctgagacct	ctggcctccc	900
gccagcactc	cctgtccgag	gagcccgaga	tccgggcctt	cgaccccgac	gcggcggccg	960
tgagcccta	ccaggaccag	acctaccagc	ccgtctactt	cgtgtctgag	agtttcagtg	1020
acgccaagga	caagctcagg	tgggccgggg	cccggggccc	ccaaactgga	ggatccagcc	1080
tgagccccg	cctatgagcc	catttcccag	cagagggagc	tgctgcggac	cccaccgtca	1140
caacccccct	cccacagctg	gaaccccaga	aagcctgcg	aggggggacc	tgaggggctg	1200
tggccaggtc	aaggccaggt	cgaggccagg	cttttagggg	tgaagtctga	ctttgtaaga	1260
gggggtgcag	ggtccttccc	agcctcctcc	cctccgagca	gctggggggc	gggcgggggt	1320
gcgatgaagg	cagagatgac	gcagccaccc	gttcaccctc	aggaggcgcc	tctgtccag	1380
ccaggctcct	gttgtcacag	gggaaactga	ggcccaggt	gtgtgtgtgg	gggggtgatt	1440
ctcacacaca	agcttaggga	caggacata	acggcctctc	cagggcacac	agtctggagg	1500

<210> 13  
 <211> 3024  
 <212> DNA  
 <213> Pig

<220>  
 <221> misc\_feature  
 <222> (5)..(5)  
 <223> Nonspecific nucleotide

<220>  
 <221> misc\_feature  
 <222> (10)..(10)  
 <223> Nonspecific nucleotide

<220>  
<221> misc\_feature  
<222> (62)..(62)  
<223> Nonspecific nucleotide

<220>  
<221> misc\_feature  
<222> (103)..(103)  
<223> Nonspecific nucleotide

<220>  
<221> misc\_feature  
<222> (110)..(110)  
<223> Nonspecific nucleotide

<220>  
<221> misc\_feature  
<222> (168)..(168)  
<223> Nonspecific nucleotide

<220>  
<221> variation  
<222> (438)..(438)  
<223> polymorphism C:A

<220>  
<221> variation  
<222> (443)..(443)  
<223> polymorphism A:C

<220>  
<221> variation  
<222> (455)..(455)  
<223> polymorphism G:A

<220>  
<221> variation  
<222> (503)..(503)  
<223> polymorphism G:A

<220>  
<221> variation  
<222> (525)..(525)



<223> polymorphism T:C

<220>

<221> variation

<222> (528)..(528)

<223> polymorphism G:A

<220>

<221> variation

<222> (571)..(571)

<223> polymorphism A:G

<220>

<221> variation

<222> (636)..(636)

<223> polymorphism G:T

<220>

<221> variation

<222> (710)..(710)

<223> polymorphism A:G

<220>

<221> variation

<222> (867)..(867)

<223> polymorphism C:T

<220>

<221> variation

<222> (903)..(903)

<223> polymorphism C:T

<220>

<221> variation

<222> (1148)..(1148)

<223> polymorphism C:G

<220>

<221> variation

<222> (1305)..(1305)

<223> polymorphism C:T

<220>

<221> variation

<222> (1320)..(1320)  
 <223> polymorphism C:T

<220>  
 <221> variation  
 <222> (1400)..(1400)  
 <223> polymorphism C:T

<220>  
 <221> variation  
 <222> (1519)..(1519)  
 <223> polymorphism G:A

<220>  
 <221> variation  
 <222> (1547)..(1547)  
 <223> polymorphism T:A

<220>  
 <221> variation  
 <222> (1607)..(1607)  
 <223> polymorphism C:T

<220>  
 <221> variation  
 <222> (2222)..(2222)  
 <223> polymorphism G:A

<400> 13  
 ttaantccan gttggcccga caagttttcc ccatttgaaa agggggccagt taagccccaa 60  
 cncaattaat tggaagtttag ctccccctcat taggctcccc agnctttach ctttatgttc 120  
 cggttcgtat ttttgtggga attgtagcgg atacaatttc tctcaagnaa ccagctatgc 180  
 ccatgattac gcggtacagt agttcatcag tcccccccg ccatgggaca gcgaaggga 240  
 ccagtatgtc gtggggcccg gtctaaagg gtcaccacca gggaggggca ggggctccag 300  
 gaggcagggc cactgagcgg tacctggtgg ggggaggtgg tggggccaca ccaggagtc 360  
 ctgtgcccc cccactcccg ccgttgga tgagaagcag gggccagcct gcgggtccct 420  
 gagttcagcg ccccccccc ccaccgccg agcagcccg ggtctcagca ggctgctgtg 480  
 ctgggggccc gggcgcttat ggrgccggga gcagcccccc cccacggctt cagagcatct 540

ctggggcctc agggatggac cggggctctgc rggcaggtgt cctctcgcg cccactccc	600
tgggctataa cgtggaagat ggggccaag cccggkcggt ttggcctttg tccccagcca	660
gtggggacag cctggccctc aggcgctcg ttaagactct aatgacctca aggccccag	720
aggcgctgat gaccacgga gatgatcccg caggcctggc agcagggaaa tgatccagaa	780
agtgccacct cagccccag ccatctgcca cccacctgga ggccctcagg ggccgggcg	840
cggggggcag gcgctataaa gccggccggg ccagccgcc cccagccctc tgggaccagc	900
tgtgttcca gccaccggc aagcaggtct gtccccctgg gctcccgta gctgggtctg	960
ggctgtcctg ctggggccag ggcctctcg caggaggacg tgggctcctc tctcgagcc	1020
cttggggggg gaggctggtg ggggctgcag gtgcccctgg ctggcctcaa cgccgccgt	1080
ccccaggte ctcaccccc gccatggccc tgtggacgcg cctcctgcc ctgctggccc	1140
tgctggcset ctgggcgccc gcccggccc aggccttcgt gaaccagcac ctgtgcggct	1200
cccacctggt ggaggcgctg tacctggtgt gcggggagcg cggttcttc tacacgcca	1260
aggcccgctg ggaggcggag aaccctcagg gtgagccgag ggggygtccc gggagcgty	1320
gggggagttt ttaaggagga aattggtaaa agtgaccaac tccctgggag ctgagcccag	1380
agacacccct cccacgccc ggtcccgctc gagaagccc ccttccctcc cctcctccc	1440
aggcggtcc agggaggaat cttacggagt caaggccgg gtgccgctgg tctccgagt	1500
acatggccgt ggtgtccrt ctgccggccc acatgccgt gagagawgc ccatccccct	1560
gggagggggc cccgtgccg gcaggcggc ggaggcccag gaccggtggc tgctgcggct	1620
tccactccag ggtgggccc gtggggggtg gctgtctctg tgtgaccggc tctccccga	1680
gcaggtgccg tggagctggg cggaggcctg ggcggcctgc aggcctggc gctggagggg	1740
ccccgcaga agcgtggcat cgtggagcag tgctgcacca gcatctgttc cctctaccag	1800
ctggagaact actgcaacta ggccggccct gagggcgct gctgctccc gcacccaaa	1860
accaataaa gtcctgaatg agccggccg agtcctgtgg tctgtgtggc ctggggcggg	1920
ggccctggtg ggggagggg cagaaggctg tggggggcct gcctgcgacc cctctctgct	1980
ctcgccacat cggctgtct aagcttcctc cacatgcac gggtgccac aggcacatgg	2040
gcaccggggg accaggccc agggcagggc cttcaatgt ggcgagctct ggttttcagg	2100
gctccagaca cccctcctg ggtgccact gctgcacagg gtcactctga gggtcacagg	2160

gcacccaccc agactgctct tgggcacaca aaatagccca ggggcttctt gggctggctg 2220

crgtctggga ggtcagagag tgaccccgcg ggaccaagac ctggccagcc tgccagtcgc 2280

ccaggccaaa ccaatctgca cctttgctga aggttccacc cgggccagca ctgggggcg 2340

ccgggcctag agctggggcg cggggcccca gggactgcac acccgccaga ggtgggcctg 2400

aggggtggca gcaggtctc cgctgggac ccagccagct gggcagctca cctctcaaca 2460

cgaggctctc acctgtgtcg tcccctcccc acggccacac agacaccct ggggagaagt 2520

cacaggcccc cagcaggccc cgcccctgga gaggaggcca gggctgggca ggcgggtggc 2580

cggccggaca ctggaccgga aaggggggta ggcggctggg atgagtggcg agctgtccat 2640

gggagcacc cgcggcccca ttggcaccag tacaggcagg ggcacctgca gcagctgagg 2700

tacgtggggg ccccggaactg gttggtgtcc ggctgccctc tgggaggcag cgggctgagc 2760

ttgtggctct gcccaaccagg gagaccctg accaccctgc tgcttccct cccccccagg 2820

gccagcagac tcctttggga ctcggggcc ctgagccgcc cccactcgca ggactcacgg 2880

ggtgtgcgg cctgggtgag tgggggcttg ggagagggtc actcttgctc gtcgggtggg 2940

gaaggctgag agtcatggtg tgacagcgcc ctcggcctgc cgggtggggg gtctcccttc 3000

tcccgagccc agatccccgg gtac 3024

<210> 14  
 <211> 1730  
 <212> DNA  
 <213> Pig

<220>  
 <221> misc\_feature  
 <222> (530)..(530)  
 <223> Nonspecific nucleotide

<400> 14

cgtaaccgc agaagccagg cccacaggcc ttggctcagc ccctccacc aggccacgt 60

tccgcccctt ctgggaactg gaggacagcc cgccctcgcc ctcggaactg gcttcgtttg 120

ccctggcatc tggcagtggc cggcagctgc gttcagccct ggatgacacc ctggcgtgag 180

cgggtgggtcc ccgtgctgag ggcagcccc acacacgtcc tgctcacttg ccttgtgtct 240

gctccgcac cgtcatcac acatgccatg ctggggcacc gtagcgctt gccctgtgtg 300

gcactgtggc actgtgttcc tgatgggaag actgaggctg gggtcaggcc cgctgctgcc 360  
caccctctaa ggacattctg ccggtgcagc tgccctccagg ctggccccc ggattgcac 420  
tgcttctggc acggatgaac tggcacctct gctgaccat tagggctgta tttgccttct 480  
cctgttggca gtaaataatt actgtccctc cctgttcctc caggcccgan ccagttcctg 540  
aggggcatgg gaggtggaca caaagggtgcc caagcagccc cctgctcttg agggcccagt 600  
gtctggtggg ggccagcctg ggaaggagga gcgagactag gaaccagagg cctgtgttcc 660  
tggaaggggc cccctggcag agttccggct ggtgtgtgtc cagctaggct gtgagtcttc 720  
aaactgggga gcccgggccc tggaccagc cagggtgca cccctggtgc cagtgttca 780  
ctgggtgggc acctgtccc accaggcaag gtggtccgag cggtcattca cagacagaac 840  
cagcagaggg cgccaaagcc ccacttttga caaactcccc ttcgccctga gccgaaagtc 900  
caggcggcag gtggacctct ctgcagggt ctgccacccc tgctgccgct tgccagcact 960  
cacaggggct gcggggggtg cccaacaggc cggctaccct gagctctgga ggcgatggag 1020  
tttagggagg aacgagggga ctctggggg tgactttctt cagcgccac attgcgggcc 1080  
agcaaaccga ggctggagga ggccgggcac ctgtgcccag ctggagcctt tgctgagggt 1140  
ctccaaggcc tggggaaatt gaggtgggg gctgggggt gtcactgtcg ggccaggagg 1200  
ccctcgctc tgattggagc cgcctcgccc acttgagcca ggaggctcac atgaggcggg 1260  
ggctgcaggg acaggaccct cggggcccgg gaggccttg aggggtcca gctggggcag 1320  
ggttcgttct tccccgggtc catgtccacc gccctccgc tgctgggagg agaggaggtc 1380  
cagggcagaa agaatgcgtg gggatggggg ggtggtcagg ggtctgggag ctgtggaaac 1440  
aacaacaga cagcagggtc ctggggcgcc cggcccccg cccctccgg cactgttgtt 1500  
tctggccggg gtgcagggac agcaggcag attccttcga aagtggagac tggcggggg 1560  
ccctcggggt cctcagctca cccctgagc tagcccgccc actcggctcc aacctccgc 1620  
agggccctgg cacggtctcc aggagtccac tgaggggtcc ccaaagctgc caccaggagc 1680  
tgggcctggg tctgtcacca cccacccca cctccaagt ctgagatatg 1730

<210> 15  
<211> 4833  
<212> DNA

<213> Pig

<220>

<221> misc\_feature

<222> (127)..(127)

<223> Nonspecific nucleotide

<220>

<221> misc\_feature

<222> (519)..(520)

<223> Nonspecific nucleotides

<220>

<221> misc\_feature

<222> (545)..(545)

<223> Nonspecific nucleotide

<400> 15

atgtgagctg cacagcatga gccctcggcc cactgtgtgt ggccttgccg acattgaggt	60
gtgtgccgcc cagggcgacc acaccctggc ctctcagggt gcccgtagag aggcgggtgg	120
gtcgtangag gtgcggggct ctggggaccg ctggtgagtt caggacgggg gtcatgccac	180
ctcctctctg aaggttttgt gaggtggccc ttctcttata gtgatgacaa tactgatttc	240
tggaagagcc aggtgttttc tgaggctgtg gttgcacttc tccacgtggc cacaaggtgc	300
cgggctcggg tcagatttga gaagccctgc gggagcgggt gtcatgcgcc agattcagct	360
tgcctcctgc ggggtctggg tcaggacgtg gtccccagca gtctgtcca gacgtgtca	420
gtgatgtgtg ggattttacc gctagaacac agtttctctt gattctcaga aaccagcaga	480
tgcttttaga ggggcgtgca ggtttcacct gtgtgtgcann gcccctgcc acctggtcgg	540
agccncaaga cggcatctaa agatcagttc ctcatcatca gttccgcagt gctggggtgg	600
gggcagatga gaacctcagg gctgggcgca gaggtgggga gcccgctgg accccgacac	660
tgcagggggg cctccccctt gtaggaagaa caatgtcgtt ttgccacca gccctctccc	720
cagggtgccc cgaactgttg ctctaagac ctctgggctg tgtgtgttaa ttctataagt	780
ggccaccagg tgtcagcagg aggccactta agcatccatg tggcggaac ctggagctgg	840
gggttcctaa gggtcctcgt agtgtctcct gaataaatag gcgctgacct gatccccagg	900
aagggataac cctctcccag gcctaagagg cagtggggca atgaggttta tgtgtccact	960

gtacccccaa attgtctctt ccttccctct accctgtgtc cccaccgtgg acgatacacg	1020
gagtgcgagg ctgcgggtca cagccctcac agccccaaag ctgcagggtcc tgcctcaggg	1080
gcaccgcage ttggctgggt ccccttgggt cctccccacc ctgaccgtc ctctgctccc	1140
ctccctttgc ttaaattgtc tgcgtttcaa ggttctgatg gaataaaata gccctgcact	1200
ggtgtgttcc tctttggggc tgtgccagaa gtgggaattc agaccagggc agagctcaga	1260
ttccacatac tgtgttaggg atggcagggt ccacatttcc aggagtttca ttggtggttt	1320
gtaaatgcta cttccgtttc agcccctcag ctgcccacct cctcaattta gggaccccc	1380
cctttggcgg gttgcccatg gaaccacatc atctggcgtg gggtagagccc tttatcctcc	1440
ctggccccac tgggaggggt tggggaagtc ccagctaaat ttctccgtag ggacctggaa	1500
ggagcccttg tgacatctgg gcacagataa gaggtagggg gcacaggccg tgaacacttg	1560
aagctgcaga gccagagca gagccagcag gagcaagtga ctgctcccca cccaagaac	1620
tgtgggctgc gtcacacact cccactgtg tgccctggac ctgacagggc ctttagcctc	1680
cctgcatccc tccccacca agaaccaggt gaggcacccc acttgcccct ccttagtggt	1740
gttatggctc tggggcatct gcattttgtt taggacaccc ccagctagat ttaagtcccc	1800
ccaagtgtga ctctttctc cactgaaaac cctgtcctcc caccaaaggg ccctatccct	1860
ttagctgagc caaggaaatt caggaggggc cttgaatgac aaaggaagag ggggagagtt	1920
aaacccaac actggctggc aagctgggtg gggtaggacac ccagggtgc aggggtgcag	1980
tgaaggtagc ggctggtggc cttctggaaa ctacatgtga ctttgccatt aggtgagtct	2040
ttgctttgcc cctgctctat ctgcaggctt atggaagaag tttaaattcc cagggacact	2100
tggtctaacc aggcagcgt tgtatctggg cccttcccca gctgctgacc actctgagtc	2160
tgcgcccttag ttggagtttt ggccaagctc aagaggctgt ggaccccagt catccccccc	2220
aggggtgcct gtgggcagga cgctgctgcc tgccatttgc tgcagtattg tcaactgtccg	2280
gcaccacaca catggtgcag ggggtggtat cagggtccac tggggaaggg agaaaactcc	2340
cagggtgagtc ccctgcctct ggaagcaaga tggacatgac cgactgtgt tgcagctgca	2400
ttgggaggcc ccgaagaaag atttttctga tctttctcga accctgcttt tccccatcat	2460
gccccgcccc cattttaccc gtgccacgcc cactggtgtg ccgggggtgtc aagtgactga	2520
caagtgtcaa tctactgagg cctgcccac tctccacccc cccacatagt cccacctccc	2580

agctggcagg gagaacttcc agctaattgcc catgcccaca aatgtctttc tgtcagccta	2640
gagctggacc aaatctccac cctgtaacat gctgtgccct ggcgtgggaa ggtgccagag	2700
ccagttgccc cagcagcccc agaaccacta agttggcaca aagctaccca aatttgagg	2760
ggcttgggga agggcatgga ggggatgagg aggtgagggg caaaactaat ttcagttagc	2820
atttgagcag gtgccacgct cagcgtggag aggtctctct gcttctaggg acccattatg	2880
atgcacacgc taaaagcgcc cttcaccatc tctccagcct cagctttgtc cccctcctcc	2940
tcctcagcgg caaccgggct ggaggggtctg gccactacag ccagagcgcc ccctactttg	3000
gtggcgactg ctactattgg cccaaccagc ggatcaccgg ccaggcagtt tcggcagaga	3060
gtctggggca ccagtgactc ccccgctctc tttatccacc acccaggagc ttcagggact	3120
acacagcgac tagagggcag gtaactggtc tgccctccct agggctgccc cctcagagt	3180
tgtgagaaaa gctgcattga gtgtttgggt gcagggtgggc tgggggcttg gggcagcaa	3240
caggaacggc gggacctctg cttccagagg accccagatc ctggcaagct tcgactttg	3300
aggggacagg aaagacaggt ggagagggga cacttccctc ttctgtacag acgcccaccc	3360
ggagccacag aggccttttgc aaggaaaata ggttttccct cactaatgca gcaggcaaaa	3420
tgggaggggc aggggtggag ggtagtggcc ccgccccag caggagggca cagctgtttc	3480
tgcaaagtga aaaaagcagg gtttttctgt gtgagaagtt ccctcttgct gcatgtcccc	3540
acccccgcca ccaaagacaa acaggacact gtgcagaggg gccagagccc cgagattttg	3600
gagttgtttt tatatgcata tataccattt tgaaagcaaa gcttccctct cccctactcc	3660
ctacatgtcc cccttcacca aaaaatccca ccacgtaact ggaaagggga gtgagaagga	3720
cgacgaagg gactgtccc ctcccgctcc acagcgggac ttaaaacgta cagcttttcg	3780
cctccggaca gtgtgccgc ccctggcccc cgtcacgctc ccctgcccgg ggggctgagt	3840
gtggggccag ggctgtctc caggcatgca ttattttgtg catgaagggt ttgtcccgcc	3900
caccagggct ggtgttgggg ggaaggggtt cattgtcca aagaagcca tctccccct	3960
cagccacctt cagccgcctt cgcaaggcag agctgtgtcc tctgctgtgt gcctggcccc	4020
ctccttgctt ctattcaagg tggaagtgtt ggggggagga gaagagtttt tatattgtgt	4080
ctgtgatccc ccgaggcagg gcatttgtgt gcggcccccc agccccagc cccaggcaga	4140



tgggccagcc tgcccagacag aaggggtctcc tgctgcttgg ctgcagggaa acccagctct 4200  
 ggggtgaaccg tgggcacctt ctttctctcca tgccctgtat ttaaagaagg agagctgggg 4260  
 ggccagagggc acagggaggg gagccacggc cccaggtctg acaagatgac ctgcgggcct 4320  
 ctccacccaa gagtcgggggt gggggggcgg atttggtttg aaaagagaac aaataggaac 4380  
 acactcttta ttttccccag gggccgaaga gtcacccctg aacttgagga cgagcagccg 4440  
 gattccagcc cccagcccca gggccccaca tctcctcggg ctccagccgg cgccccagct 4500  
 gccccccagc ctgagctgca gcaggccagg gctgcccgag accccagccc ccaggtgagc 4560  
 tgctgcagcc tgtggcccag gagatctccg ccggctcaga actgaggcgg gcagcccacc 4620  
 cagcccacag cggtagagtgt ctccagaccc cagggcaggg cccggtgtcc cccggcacag 4680  
 agagctgtgc tgcaggccca gacctccag gccgttttag ttcccatctc cccttggggg 4740  
 aggggtgggg ctccagaggg ctgggggtgca tccgcagagc tgggggtgcag ggctccaggt 4800  
 gcctctctcc caggcggctg gcccgaggg ggg 4833

<210> 16  
 <211> 2064  
 <212> DNA  
 <213> Pig  
  
 <220>  
 <221> misc\_feature  
 <222> (46)..(46)  
 <223> Nonspecific nucleotide

<400> 16  
 ctgggtttcgc actcctccgg ggactgttga agtaccagag agcgcnccgg gagcgccggg 60  
 gcgagcgggg gtggccgccc ggggtgctcc cgggcccccg gaccgagcca gggacgagcc 120  
 tgcccgcggc ggcagccggg ccgcggcttc gcctaggctc acagcgcggg agcgcggtggg 180  
 gcgcggccgc tgccgggagt ccgcctgcct cctcggaggg gcccgaccgg ggagcctggg 240  
 ggaccccagag cggccgggga gcagcgcccc gacacgcccc gggccgctct cggcttcctc 300  
 ccttccagcc ggcgcccgcg cggccggggt tcggcaccgg ggcgctctca gtggcaggag 360  
 aagcgtgcgc tcccgcgggg tgggggaccc gcaggaaacc cgcaccgcct ggagccgccc 420  
 ccgcgcggcc agcgctcgcg tccccgggg agggcgccac tgctccgcgc gcgcgtcccc 480

cgacgccccg cgcgcttccc cggccggccc gggatcctaa cctctctctc ggtcgcagcc	540
ccgcatcccc aggggtccag gccccggcg acttgccgc tctcccaat tgcagacacg	600
actttttctg ggacctccca aaggacagcc tgggtccagg gtccccaga tacattcacc	660
atctctccag atcacaagtg ggtttttcgg gcactaactt ccagagacct caaagcacat	720
gagccccctac tggctttccc aggtttccac tagtggcctc ggtecccacc tctactgggga	780
ttgtctccca ggctcttcgc ggtgtgatcc caccattcg cggccaggtc ccgcagtgcc	840
aatccctcct ctagaaaact taaacactga ctctgtgtct cggggtgagg ctgccaatg	900
tgctgactc cccagaaggc ataccagtgt tttctggca tttgggcacc gttcccccaa	960
aacacgtgaa gctcttttcc cgcgtcccca taattttgga cggcaggggc acccaagctt	1020
agcgcacctg tttggctccc ccacaccgc aagccctgct cctgggggtt cagcacagtt	1080
tgggacttta tctgccaagt tccacaaact gattggcccc aagctggggc ccctaaattg	1140
tacacaaaga accccagccc cccccccaa ctccagtaca ggaagcgatg gcccaggga	1200
ccctcggagt tggaacgtgg cttcctaagc cttcaccaaa attgaggctt tccgcgatg	1260
gcgcgctgat gcccttgctg aatcagaagc actctgccct ctgattcctg ctttccacaa	1320
ccctgagagc atgatttctg gtcccccaa ctcactgagc aaaaatcttt ttgtgggggc	1380
tgcaaagata ggaggcattt ctctccggag ctctccaaac tcccttgctt ataataagt	1440
tccctaaaac ttagacagag cttcccaggc cccagaggca cacagagcca ttattggagc	1500
tgcgtttaat gatgacaggc accatgggtc atgcagctcc cccaagtcac aaatgcccc	1560
ggatccttg gctccagcca agcccaaagc aaactcttg acagatcca tatcttgta	1620
tgtcaagcgc tttgcgtgct ccagtaaaca aatagtctga gtgttttctc cacctcataa	1680
cattcggaat attaaaaaat tccctgggccc cccggagctg acagacaaga atccgggctt	1740
cctaaaattc agaactgatt cccaaatccc aggccaacgc cagacctct cccaatctgg	1800
agcccctccg actggacaca ctggactcct aagtattacg cgctgtcctc caggcacc	1860
aaatgcattc aaagtgacgc tttggtcaca gaaaggcact gatttcttgg gctccaaagc	1920
agcccatgca ccccgagtc accccaaact tagtcagcat tccccgggtc tccctccgca	1980
ctgcaaactc ccaactgcgg acaccggttc ttcaggacc accgcctaga cggctttaat	2040
cccttttccc ccagacctag attc	2064

<210> 17  
<211> 371  
<212> DNA  
<213> Pig

<400> 17  
agattcaaaa actatTTTTtC tggggcctcc aaattgaggt gctgcctgcc agtcctccaa 60  
aataaactga ggggtTTTTtT gtttgtttgt ttttttgttt gtttgTTTTtT tttttacctt 120  
ccacgaaaca atccaacttt tttggaccat tgatttatgg gtcccttgac tttatgacct 180  
ttgccccaaag tccccctaaa tgtaggcat tttccacggg cctcccaaaa tgaaattgcc 240  
cagatcccgC cgaaaaaat atccccgggt cctggaaatC ccaggTatta caggcctgcg 300  
gctgacaccc ctcttgcta ctaaccaggT tccctgaagt ttagagatca ctacctaagT 360  
aacaatcca c 371

<210> 18  
<211> 2415  
<212> DNA  
<213> Pig

<400> 18  
ccaaaactgg ggcctatct tactagggtt ccctaaatgc agacagcgcc cgggaaaata 60  
ggggcgTTTT ttttcctgtt tgccaaaaat aaactaattg aaaccaattt ttagaattaa 120  
aatctaaaat gaccttgatt ttctgcgttc tccaaatgta cttttcacag ccaggttgc 180  
ccccagttta gacggtgttg cttgaatctc taaagcaccC tgaggatttt tcccaggaa 240  
gccaccacaa ctacggaatt tactgtcctt cggggccaca agcctccagg ccaccaactt 300  
ggatttctaa accgtggaaa tcagcctcca ctccctccg ccaccccgag ggtctgctca 360  
gaccccccaa acgtgcccgc tgttcttctc ccccaaatt ttatttagag aatatgcctc 420  
tctcggttc tgccaagttt ccgctgaga ctctctcggt catcccaaa tctcttccc 480  
cacagtccgg gagccccac aagcttaccg acccatgc tggggtcccc caacttaaac 540  
gcatccct gtccccaga ttaccgagt gatttcctg gtctcagac tgggactctt 600  
ttactggagt ctgaattta gccattaatC acagttctcc actccgacgc aggtccctt 660  
gggtccccac gtcggggaca tgggttctct tgctgcaaa tcaggctgct ctgacttgca 720

ttcaggcctt tgggcattgt tccccgcccg ccgcggtctc gggtctcccc ccatcccgcg	780
cacgacgggc actgggtctg ggcctcttgg tgtctcctac aagtccccgg agctcctcgg	840
acttgggaac tgtctcttgc gttccccaaa tacactcggc ccggcagtgt gtccgccagg	900
acgtaggcag agcttctccc gcgtccagga aaacgactgg gcattgcccc cagtttcccc	960
caaatttggg cattgtccct gggctctcca acggactggg cgttgcccc ggacactgcg	1020
gactgcccc ggggtctcgc tcaccttcag cgcgtccacc gcccgtgca gagcgctcgc	1080
tctcgtctc tcggctcca gcgcgcttgg ggacgcagcc tccgggcctc cagccttgcg	1140
gtgagctccc cgtcgcctcg cgtgtcccgg cccggtccc aaaccactc gccgcgtcc	1200
cgtgggggt ggcactggcc tccggcgact gccggggaca cgggagcggg gcgcgggagc	1260
ctgctgcagg ccagcccgtc ggccggggccg cgcgcctga aacgcgcgcg gctttcgttt	1320
gctctttgca aaggtcacaa ccgtggggaa aacgcctcgg cggcccccaa gcggggcagg	1380
cagggcgttg ggaaggagg acacgcggga gaggagcacc ccgctggggc ggcgagcgc	1440
gggcctcca gccgccgggc ggaggatccc gggaggcgcg cgcggagcgc gggcgaagtg	1500
attgatggcg gagcgagggg gccagcggat cgcgggcttc cgcggcggc ggcccttcc	1560
cctcggaggg actcgggcgg cccgggtttc tgggggcggg cggggcgcgg gggcttgtgc	1620
gtggtctcca cttggtaaaa atcacaacga ctttttacgt cgcctcgact ctccaggaga	1680
tggtttcccc agacccccaa attatcgtgg tggccccggg ggctgaacct cgtctacgc	1740
aaggccaacg cgtgaggac gggggaacca ttatccgat attttgggtg ggccccaaa	1800
gcgagctgct tagacgcgc cgggtgagct cggctctgca ggtaggcttg gagcgaggtt	1860
ccccgcctg ctctctctc ttcgggcagg cgcggccagg ccggccggcc ctccccactg	1920
acggcacctg gcggccggcg agacgactcc ccggttccc cgcggcaccg gggggcgtc	1980
gggctctggc tgcggctcga ggcgtcgc cgtctcgggc aggtggaggc ttcacgcgg	2040
gccgcgccc agggacgacc ccttaccocg caggctccag cgggactcgg ggccccgga	2100
tccagcgtct agccacctgt gccgcaccg ccgcgagggc ttgtgacacc taccacctg	2160
gccgccccgc gtccccccgc gcacgaatgt agggatcctg acaccccgga acctaagacg	2220
gggcccccat acactttcgt acagcgattc gggatttctc tcgaactctg cagatctgta	2280
tggcaaagtt gatggcctgc attatttttc tgataattca gcgaaagatg gcgaccagag	2340

ctatgcgcgt ctgggtttta aaggcgaaac ccaaattaac gatctggtca acgaacagat 2400  
acagcatacg ttttt 2415

<210> 19  
<211> 3752  
<212> DNA  
<213> Pig

<220>  
<221> variation  
<222> (341)..(341)  
<223> Polymorphism G:T

<220>  
<221> variation  
<222> (2247)..(2247)  
<223> Polymorphism -:C

<220>  
<221> variation  
<222> (2248)..(2248)  
<223> Polymorphism G:C

<220>  
<221> variation  
<222> (2257)..(2257)  
<223> Polymorphism C:T

<220>  
<221> variation  
<222> (2413)..(2413)  
<223> Polymorphism C:T

<400> 19  
agattccaat ggggatcccg atgaggaagc cgctgctcgt gctgctcgtc ttcttggcct 60  
tggcctcgtg ctgctatgct gcttaccgcc ccagtgaagc tctgtgcggc ggggagctgg 120  
tggacaccct ccagtttgtc tgcggggacc ggggcttcta cttcagtaag tagctcagcg 180  
gggcacgggg gcggggcgga cacagcaggt gctccatcgg tgctgccccg gtacctgtgc 240  
gggtccttcg ggatggatgg tgtgggggac ggggggcggg gggcgccaa gggaggacct 300  
ctcctccgag ggtctgagac ttcagagcgg gggcgccctg gccctgcgca gtgattggca 360

cctgccatgt gcctggctgg ggctcacacc cctgacgtt cctgcagcgt gactcgaaac	420
gggaaaccga agggacgggt ggcacggggt ggggaggcag accgtgagtg gcaggcgtgc	480
gaggggttct ttcgggcggg gtggcccagg caggccccac aggatgacag cctgtcccct	540
cctgtctctc cttgacctgc ccacagccag ggctgcaggc actgacattc acccatggta	600
ttgtggtgcc tgacgtcttg gcagtgggca tgggttcatg gactgttga ttgaaagtgg	660
aataagatgg gttgaaaacc aataagaata aaggcgcgtg tggctggcgg catctgcgag	720
aggtgaccgc tgcctccctt ggggttgggc tttgggtggg ttcccatggg tggggcgggc	780
cgccatgcag ggtgcccgc tgctggcctc agagtgttt gccgtcctca tctttctctc	840
tggccccgt cccgtcctg aggtggctg gctgggcccg cggagacctc cgctcccgcc	900
tctgtctgtc ccaggagca ggggtgacct tcccttgggc tcttgacctg acctcccagc	960
aggtggggc tcagtgtcct tacctgtagg atgggtcagg ggcgtcctgg agagagtcct	1020
cgggacaatg gggaggctgg gggcaggccc agcctgacct tgaaggagg agtgtgtgct	1080
ccccctgggc tcagccagcc gcgcttgggg ccgggagggg gtgggggacg tggctggggc	1140
aagttgtcaa gggccgcgag gctcaccccc gcccatcgct ccccatgtgg cagcctcttc	1200
tgcagcctct acttaccac cctctgaaat gggctgaaaa caccatctt ggcatgcaa	1260
agcttctctg taaaaagcgt tgctgcttct tgatgcttct gaggccctg cctgccctgg	1320
cctctgagcc ctctctctcc tgctcgttt gggggcaggg agtggcacca tagaatctgg	1380
cgctgggcct ggggagcggc ccctcgtgc caggcttccc cgaaaggagg gctgggctga	1440
gctcccgacc ctctggacct cttaccagga ccccttacca ggggcttccc ccccccccc	1500
ccccggtggc ggcgggctgg gctggggcct tttccttgca gccgagtcgg agctgtcgga	1560
ggcgagggcg aggacgggaa gagaggaggg cgtgggttct gctggtcctc actcctctcc	1620
tcccgctctc ctctcctcc tccattccc acctgtgtct ccgggtcccg gggccgcagg	1680
ctgcccaggc gcctgctgat ccattgggga ccgcactcgg gtcccgcgtg gccttcgggt	1740
cagggccacg gccacctat ttccaaaca gccttgggtc gaggcccaag aggtggggc	1800
cggtttaagg acggggaggg aggcgccaag aggccagggg ctggtcccg gcacgcccgc	1860
accgcctcac cccgctgtc cctctctctt cccggggggg cccctgtgca cccactctc	1920

acttcttctg	ctcgaggcca	cgaggctggc	tgtccccgca	aggtgaccgg	gcgtcctgtc	1980
tggagggcgg	gggcgggggc	ggctgggggc	accgtccgtg	cccgggggcc	ctgtgctgac	2040
gtgccctccc	cttggtcctg	tgggacttcc	aggcaggccg	gcaagccgcg	tgaaccgccg	2100
cagccgtggc	atcgtggaag	agtgtgtgctt	ccgtagctgc	gacctggccc	tgctggagac	2160
ctactgcgcc	acccccgcca	agtccgagag	ggacgtgtcg	acccctccga	ccgtgcttcc	2220
ggtaaggcag	cccctctctc	ggcagcgccc	ccccccgggg	gggggctgtc	tcctctgagc	2280
cgggggaccg	ggg'gcagcc	ggctcttggg	cttcaagtgc	tgccagaggg	gccttccccg	2340
ctggggaccc	tggccagaag	ccagggcagt	cttcgctctg	tcgcagggca	ggcaggcagg	2400
aggacccccg	agaggttggt	gttctgggac	aggggctggg	ggggcaggcc	ccccctgac	2460
gggccccttc	cctctcagga	caacttcccc	agataccccg	tgggcaagtt	cttccgctat	2520
gacacctgga	agcagtcgc	ccaacgcctg	cgcaggggccc	tgccggccct	cctgcgcgcc	2580
cgcgggggtc	gcacgctcgc	caaggagctg	gaggcgggtca	gagaggccaa	gcgtcaccga	2640
cccctgaccg	cccgtcccac	ccgagacccc	gcgcgccacg	ggggcgccct	tcccgaggcg	2700
tccggccatc	ggaagtgagc	caaattgtcg	taattctgcg	gtgccaccat	ccacctcgtg	2760
acctcctctc	gaccgggacc	gcttccatca	ggtccccctt	ctgagatctc	tgtacccttc	2820
tgtctgcggg	catctccgcc	ccgggccccg	tgccccaaacc	tccccatgtc	aggctagtct	2880
ctcctcgggc	ccttccatcg	ggccgagggc	atccaaacca	caaacccaat	tggtctggtc	2940
tgtatctccc	cccaaattat	gcccccaatt	atccccaagt	tacataccaa	aaattgaacc	3000
cctcaaccac	accacatac	aatcagcccc	cgtaaaacga	attggcatct	ttaaaacacc	3060
agaaaagcga	attagcttta	aaaaaaaaat	aaacccaaaa	tatcaattag	ctgaaaaaaa	3120
aataactaaa	ataaattggc	ttaaaaaaaa	ttggcaaaat	aaaagaattt	ggcccccccc	3180
ttccttctct	ttcttttcgg	accttgagtt	aaattggctg	tgacctcatca	tccaagagaa	3240
aggaagggac	caaaatttgc	aggtaggctt	gtcgccgctc	acagccatct	ccctcctcct	3300
gccacaccct	cgcgggccac	tggcgggtgtg	gcaccaagga	cccagtcccg	tcctctctct	3360
agtcccatga	ccgagaccgc	ggtggagttg	gctgggagac	cccgtgagat	cagaggaggg	3420
gagcacggaa	ccagaaaccc	aaacctgcac	aggtacaaca	tgactggccc	cccgcacagc	3480
ccaagacctc	tcatctcagt	ctccacttaa	aaagcacctg	taccacacag	catccctgca	3540

gaaacacaca cacacacaca cacacacacg cacgcacgca cacacgcgcg cacgcacgcg 3600  
cacacacaca ctcatgcgta tacacacaca cacacgcacg cacgcgcacc cacacacaca 3660  
catgcattca cacacacaca cactcgtgca tacacacgtg cgcgcgcaca cacacacaca 3720  
cacacactct ctctctctgt gggatccctg ag 3752

<210> 20  
<211> 500  
<212> DNA  
<213> Pig

<400> 20  
tggctctggc ataggctggc agctgcagct ctgactggac cccttgccctg ggaacctcca 60  
tatgccgtgg aagcggccct agaaaaggcg aaaaaaaaaa aaaaaaaaaa acaaccaaac 120  
aaacaacaaa agccaaaaca cacagaactc acagacacaa gaagagactg gtggttgcca 180  
aaggtggggg cgaggggtggg aaaaatgagg agaggggggca aaacacacaa acgtgcagcc 240  
ataaaatggg aaagtcccgg ggacctccgg tagcgcgtgt ggggactcgg gttgagaaca 300  
caccgtgatg tgtattcgcg agttgctaag agtccctggt ggagaaacaa atgcgtatcg 360  
acgtgtggaa atgaaagtta acccgacctg ctgtcgtgat cactttgcaa cacatacaga 420  
catagaatca ttatgtttta cccctggagc tgacagcggt atacgtcccc cagcctcaat 480  
ttaaaaacag cgttgccgtg 500

<210> 21  
<211> 400  
<212> DNA  
<213> Pig

<400> 21  
ttcatactgt gcaatgccag ccttaaagtgc acagaggaga gcattaactt ctttgcagaa 60  
tactgaaat gataccactc atgttttgca acttgcaactt gggcggtatt ttattgggtgc 120  
cggaacagcg gcgatgtggc accaaactag cgcgcgtgtt tttatttccc ctcggtatcc 180  
gcgctctcgc tgtcttcccc cccttcgct tgcagctgag gaaagggctg agaggaggaa 240  
agtctgcatt caccatctc cccctgcctc tgttgtcatc cttcacagaa gtggtggcct 300  
gtgcggggaa gtcactaaac ctaggcaggt gtcccggtgg gtcatgcttg ttacaccttt 360



gtgcacctgg cccaagttct ggggtggagcg agaacgtggc

400

<210> 22  
<211> 559  
<212> DNA  
<213> Pig

<220>  
<221> misc\_feature  
<222> (549)..(549)  
<223> Nonspecific nucleotide

<400> 22  
agctagcccc cccagccagg gccaggcctc tctgccacc cgcccagcca gcatgtctca 60  
agaggagggg gcctctaagg gatgaggacc tgctccagtc ggagacacga agccccgccg 120  
gtcctcctccc gaaagtccag ctgcggcttt cgagcacggc tgcgcccttc gtcaatcatt 180  
tcagccacag aagtgaaagg cgctttcgtg gccgaggcag gcgggacaca gaatggaatc 240  
ccaccccaga gcgaagagcc gccgtgggtg aagcgcgtct ctggtgggga ccgggcccggg 300  
aacttcacat ggggggtcgt gtccccatct ccccatcgtc attactgcag gggctcggcc 360  
acacccggag ctgcgggggc cagtgtctga cactggacct ggctccgtc ctatgatgtc 420  
atgggggchg ggccagcaca gggcagtggc cacacctcgg gcctcccagc accagccagg 480  
atggcagagg gccccacccc accacggggc atgtacatcc cagaggacca gctgagcaag 540  
gcttgatang ggcttcaac 559

<210> 23  
<211> 450  
<212> DNA  
<213> Pig

<400> 23  
cgtgcagggg cccgtgcggg ccttcctgtg gccacagaga acaaacacac cattatcttc 60  
agccccaccg cgcggcctgt taatgggtaa actggggcaa gggggcccct gcctgaggcc 120  
ggggtgggga gcgcaaggca tggcctgtgt gcccagccc agtccttcag ggcgctgctg 180  
tctgcaccg ggggccccag gaagcagagc acccagcttc tcccctattc tagaaccagc 240  
ccccagaacc ctggaccag acccaggccc aggggatact gacagagcca cggcaaggcg 300  
gccactccac accccacaga ggggccagca aaccccagtc actgcgcagc ccatgcccag 360

ggggcagatg ggacacgaga gcagccctca tccacagcag gcaggggagt gaactggtgc 420  
 aaaacggggc gggtccacga aagttaagca 450

<210> 24  
 <211> 535  
 <212> DNA  
 <213> Pig

<400> 24  
 tgccagagac ctcagagctg ggctctgcct tcccgggctg acacggaggg ctgtggcttc 60  
 caccacccca ggccacagcc agcctgcca agtcctgaa gtgtcccag aggtggccct 120  
 gcctccacgc ccaacatcag gcctgctgca gccctggacg gccccctgtc ccccggaagc 180  
 cctcggggct ctctcgctc gcctctgggg aaccctcggg aatgtggccc agccgtgcag 240  
 tggccggatc atttgctcag gggggcccaa ggcagggggg tgacacatcc gcaagtaccg 300  
 catatgcaca ggatatggat tgggtgtgga tttaaccttt tcgcaaagt ctctgccggt 360  
 acaaatattg tttctaatac tctgcctccc tgagccgggtg agtctgcccg ggagctgcgg 420  
 ggagctgggt tgctgaacct gccctggccc ccacccccaa gggagccccc ggccagtgtct 480  
 gagggcagga agcttgggca caggctgcag aggccagcgc tggcctcagt cacct 535

<210> 25  
 <211> 868  
 <212> DNA  
 <213> Pig

<220>  
 <221> misc\_feature  
 <222> (310)..(310)  
 <223> Nonspecific nucleotide

<220>  
 <221> misc\_feature  
 <222> (857)..(858)  
 <223> Nonspecific nucleotide

<400> 25  
 tattgaagac cctatcatga gttcccagag cggaggggtg gaagcagggg cctacagccc 60  
 actccccatc actccagacc cgtccggggc tgggtgtccc tgccccctac tcctgtctct 120

ggtgggcgga cgctcgaagg aggcactctg gcctggagcc tggaggggtcc ctgaactccc	180
gctgccacct gggccctcgg gctcctcctg cgctgggacc cgcggtggtg ggaagcagcc	240
ctgctcagtg ggaggaggca gggctgtggc cgccccgcac ggccctgggg gggacgcacg	300
caggacgcan gtgggcgtgt gtgagtcctg ctacacgtcc agccaagggc ggccgcgacc	360
ggccaggggtg ggcagcccca gcctcagcag ggcgtctctt ggggctcagg ctgcgccgac	420
gggagatgag ggggtaggcg cagtctgggg ctgctgccgc agaacctcgc ccagctggca	480
gctgggcaca gggagacctg tactcccaga acctgaggct ggacgtccga gacccgcgtg	540
ccggcctctt ggggtgcctgg tcagggtcct ctttctggtt tgtgggcaga acctcctcag	600
cgcgtccttg catggggtgc taatcacgga gtaaggagcc agagaatgag gcacggagta	660
tccagtgtta accctggagt atggagacgg gagtactaat tgtggagcat ggctctaagg	720
aatggagtat tcgtcacgga gaacgcgggg ccgggtgaaa tacggagagc ggcgtacgga	780
caacggggac ggggtatccg aaggggagga tggagtatcg gccggagggg ggagaatgga	840
cactagagga tgtatanngg gcgtcaat	868

<210> 26  
 <211> 500  
 <212> DNA  
 <213> Pig

<400> 26	
accagtttcg atgagcaatc ccagcggcgt aacattatgg ctgcagcctg gtcaatgccg	60
gtggagtttg aacctccacg cgtggcgatt gtggtagata aatcgacatg gaccagggag	120
ttgattgaac ataacggtaa atttggcatc gttatcccgg gcgttgacgc aactaactgg	180
acgtgggcgg tgggaagtgt gtcggggcgt gatgaagata aatttaattg ctatggcatt	240
ccggttgtga gaggcccggt atttggtttg cctctggtcg aggaaaaatg tctggcgtgg	300
atggagtgtc gattgctacc tgcgacttct gcgcaagaag aatacgacac gctgtttggc	360
gaagtagtat cagcagcggc agacgcacgg gtatttgtcg aaggccgctg gcagtttgat	420
gatgataagc tcaatacgtt gcatcattta ggtgctggga cgtttgttac cagcggcaag	480
cgtgttacgg cgggttaagc	500

<210> 27

<211> 900  
<212> DNA  
<213> Pig

<400> 27

atgtttgatg tccgcgcgtg ctgtaaaaat ttacgctgct cgcgttcttt ggcttcgtcc	60
accaccggaa aacggacaaa aatttcctgc ataccttttt ctttcaggcg gaagccaatg	120
tcgtaatctt cagtaagact ctgcacgtcg aaagcaatac cgtcacctgc agctaacagt	180
gcggtcacgg cgcggcggct gaaacagggt cgcacgcctg cgtgggcac ttgtccggcg	240
agggttcac gcaccggaac atctttgcc tgcagctctg aaaactcatc aatgtaagtc	300
atgctggtga agtgcgtcca ttgcggttcg aacggatata cgggatctg aatcagatct	360
ttacgctcga ccagatagtt gaacagacgc aattccatcg gtgaaatcac atcttcggcg	420
tcatgcagaa taaaaccagc aaaagcgaaa ttggcgctac gctcaaattg ggtgatggcg	480
tccagcacgt tgttcagaca gtcggctttg ctggtggggc caggacgcgc gcagactacc	540
ttatgcacat tcgggaagcg agcgcacact tcgtcaacat cacgctgagt atcggggtcg	600
ttggggtagg tgccaacaaa gatatgatag ttttcgtagt cgagcgtggt cgcgcgcagc	660
tcggccatat tgccgatgac gcccgtttca ttccacgcgc gaaccataat cgctaacggt	720
ttttcatctg gtttatacag ttgcgggtaa ctcatctcgc ggtagcggcg ataaacactc	780
aacttgcggt taatgcggcg taccaggtat acgacatcta taaaaaatc gtccagcccg	840
ctgatgaaca tgatgaccgc taacgttatc gcgattactt ttaagccgta tagccaggta	900

<210> 28  
<211> 500  
<212> DNA  
<213> Pig

<400> 28

agctggatgc cccagctgt ggtcccttcc ctccctcag ggcaggttct gtccctcttg	60
cagccaccgt cactgctgtg gacaggtctg cacaccgcgc gtccaccaag agcgtggcag	120
gtccctgggc acgggcccgc tcctgacgca ccatgtgttc aaggcaagag cactggacag	180
agggtccaga cgtcccttg tcctgctcag gcctgggcgc gggcagccct ggccgggagag	240
gccctgggca tcagagcctc tgtggcctgg agcttggcgc cctgccctcc ccacctccgt	300
cctgctctc gccgcgtgc acggacctct cccggcccc caggctcatt actcttaagg	360

accctagccc cctatgctga aatgctgtac ctcgtgcttg ttttcatctg tttattacct	420
tatcttcatt cctgcttgat gatctctggt tattctttat tgattatata tatcttggtc	480
gtgtttttat aggacactgt	500

<210> 29  
 <211> 450  
 <212> DNA  
 <213> Pig

<400> 29	
agtgcggctg ggccgtcctg acgctcaaca ccgtatttcc acgcgaccgc ggattcaacc	60
tggtcacacg gacgccatgt agacatgttc ggggttacgc gcagagaagc gacctgctca	120
accggctggt gagtggggcc gtcttcgccc agaccgatgg agtcgtgggt gtaaaccatc	180
acctgacgct gtttcatcag cgcagccata cgtacggcgt tacgtgcgta ttccacgaac	240
atcaggaagg tggaggtgta cggcaggaag ccaccgtgca gggagatacc gttagcaatc	300
gcggtcatac cgaactcgcg aacaccgtag tggatgtagt taccgcgagc atcttcggtg	360
attgctttag aaccagacca cagggtcagg ttagacggcg ccgggtcagc agaaccgcgcg	420
aggaattccg gcaacagccg gacgaacgct	450

<210> 30  
 <211> 450  
 <212> DNA  
 <213> Pig

<400> 30	
tcaggccaat ctgtctggtc tccaatgggg acaatttggt tctttaggct tctgtccaat	60
ggtccgaatg gccactccc cgggcgcgcg ccaagggtcc tctgtgcctc ggggtgggctg	120
gcacggaccg cccccagggt cgtgccagcc ccgtcaccgg ggcccagaag cttcggggcct	180
ctagctggct agtcgggctg ctgtgcaggg gggctgcgct gggggcagag gcgggggtga	240
ggtaaacctc ccagccgccc ggggtccctg ccgcagccct aggcgccgag acggtggctg	300
ggtcgggtacc gccagaccgc agggcctcgg ggcccgggtg accccagctg tcgcacacgc	360
tcgcagctct ctgctcacc agggctcacc cctctggacc tctcctactg cccacacctca	420
ccccgcctgg accccatgaa gccccgcgga	450

<210> 31  
 <211> 600  
 <212> DNA  
 <213> Pig

<400> 31  
 taaaactagc tctagtagaa acatttttatt taaaaataaa aaacctgact acgtcgggag 60  
 ttcccgttgt ggctcagtgg ttgacgaatc cgatgaggaa ccatgagggtt gcgagttcga 120  
 tccctggcct cgctccgtgg gttgaggatc cggcgttgcc gtgcgctgtg gtgtagggtg 180  
 cagatgagggc tcggatcctg cgtggctgtg gctcgggtgt aggccggcgg ctacagctct 240  
 gatgagaccc ctagcctggg aacctccaca tgccctggga gtggccctag aaaaagggca 300  
 aaagacaaaa aaacaaaaga aaaaggaaaa taaaataaaa aagactatgt aaatgaaatt 360  
 aacgactgcc taggggtggga ttacagcat gggaagtaca gcatggccgt gacagtgcaa 420  
 gggtgaggcg ggaaaatgga aatagggttag gtgagtttct cctgctatct gtgatgtggt 480  
 ctgctatcgc ttgaagacgg actgcagtga gataaatatg tacagtaagc atccgaaaaa 540  
 ccgccagaac ggcaaacga atgactccaa gtaagaacct aaaagagaaa aggaaataat 600

<210> 32  
 <211> 450  
 <212> DNA  
 <213> Pig

<400> 32  
 gcgcggggcgt tccggctggg gtattttaacg tggtcaccgg ttcggcgggc gcggtcggta 60  
 acgaactgac cagtaacctg ctggtgcgca aactgtcggt taccggttcg accgaaattg 120  
 gccgccagtt aatggaacag tgcgcgaaag acatcaagaa agtgtcgctg gagctgggag 180  
 gtaacgcgcc gtttatcgtc tttgacgatg ccgacctga caaagccgtg gaaggcgcgc 240  
 tggcctcgaa attccgcaac gccgggcaaa cctgcgtctg cgccaaccgc ctgtatgtgc 300  
 aggacggcgt gtatgaccgt tttgccgaaa aattgcagca ggcaatgagc aaactgcaca 360  
 tcggcgacgg gctggataac ggcgtcacca tcgggccgct gatcgatgaa aaatcggtat 420  
 caaaagtgga agagcatatt gccgatgcgc 450

<210> 33  
 <211> 450

<212> DNA

<213> Pig

<400> 33

ggatggatgct ggcgatagcg tcatcctcgc ttatgccgtg cagcgggcaa ggataaagcg 60  
cgcgataaac atgacccggc atcagcccca tgcccgcaga gtacggattc accttgccgg 120  
tcagcgccag cgtgtaatgc gtgcgcccgt gatacgcgcc gctaaaagcg atggtgccgc 180  
tacgtttggt ggcggcgcg gcgattttta ccgcgttttc caccgcttcg gaaccggtcg 240  
taaccagcag cgttttcttg gcgaaatcgc ccggcacctt ctgattcata atctcgaca 300  
gctccagata cggtcgttaa gccagcacct ggaagcaggt gtgcgacagt tttttcaact 360  
gcgcttcac cgcgccacc accttcggat gcaagtgcc ggtattgagc accgtaatcc 420  
cgcccgcgaa atcaagatac tcacggcctt 450

<210> 34

<211> 500

<212> DNA

<213> Pig

<400> 34

acgtgaggtt tgggggagga aagcggggga cgagcagccc gagaggagtg ggggctggcc 60  
tgtggctgat gaaactctga gaaggttaag agccccatt tttgtcttcc tcttttttat 120  
tatggaaaat tccaaatgga tgcaaaagtc ccaaactaa ctggacatct tcttggtacc 180  
aggaacggtc aggcacttat gatgcaccga gccccgaggg aaaaaccctg ccgtcctgga 240  
gccacggtc cagcagggca cacaggcccc agcccgaag cggcacggct gagtcagtga 300  
atggcgtgcc ctctggtcaa ggacgggcac tctggacccc aggaagcct ctgaggagcc 360  
cccttcacag cgtcaaaaac tgttaacagg gccatgttcg ccccccca cacacgtggt 420  
tcagaagcag accccaggca tcgtaatatg tcatccgtga gttccctgtg tgccaccaac 480  
agaaagccca tcgtcacgtt 500

<210> 35

<211> 400

<212> DNA

<213> Pig

<400> 35

cggcatcgat gtacatggta cgcaaggcac tcgtaaggcc ccgagcctct aggccttgtc 60

attgtcacgt gctgctcgcg gggatcagca gccaggcttg tgaccccggc cactttgaca	120
gataaggaca cagagaggcc acagcactgg tgtgaggccc cacagccagc agcccagggc	180
agggaggact ggggtctcacc tgctctagct gggcccagcc tccctgggag tcccggagtc	240
tccccagctt aggagtgtcc ctggaacctt cttctctccc cttcccggcc tcaccggagc	300
cccctgcctc cccccacca acccctccc cctccttctt tcaccttgag ctcctctctg	360
aggacctcta ctgttctgc ttatctccc ctttgagcca	400

<210> 36  
 <211> 500  
 <212> DNA  
 <213> Pig

<400> 36	
tggcggtgaa ctatgtcgtg cgtgaagagc atttgtggtc ggtagcgcgt tatatgcggg	60
aagtttaggc gaactggaca gcctgggttt atccggtagc gaaatccgct ttcacggtaa	120
aacgctgcta gcgctgggtg aaaaagcgca gacattgccg gaagatgcct taccgcagcc	180
gatgcttaac ctgatggaca tgccgggtta tcgtaaagcg tttaaagcga ttaagtcgct	240
gattactgac gtgagcgaaa cgcataagat cagcgccgaa ttgctggcat cgcgtcggca	300
aatcaaccaa ctgctgaact ggcaactggaa actgaaaccg cagaacaatt tgccggagct	360
gatttccgag ctggcgtggt gagctgatgg cggaagcatt acacaattta ttgcaggaat	420
atccgcagta aaatcttccg aagccggact gggcgcgctc agcgccacat ccggcttcgg	480
caaactacaa atccaacacc	500

<210> 37  
 <211> 500  
 <212> DNA  
 <213> Pig

<400> 37	
gatttcacaa gcctgaccca cgcggaaatg cgctaacagc gtaaagtcgt gcggccagaa	60
ttttttcgtc tcttcgcttt gcgtaattc aaaagtcagc gctacgcat cagcatcttc	120
atgatgtgat ttcagcgtcc acggcaggtt gcgggcaaaa ccgtgcgcag gcagaccttg	180
ttgtgccgcc ggaccaaacc acggccagca aaccggtacg ccaccgcgaa tagcgacgcc	240



atttttgaac ggtgtgttgt tgctcaacca cagaacttct tcttcacccg caggtttcca 300  
 cgagagaagg tgtgcgcct gtaatgcaaa agaggctttt acctggggat gatcgaccac 360  
 aatgaggtcc agttcatcca gtttacgacg ggagaggaca ggggagattt gttcgatgac 420  
 cggaagggca aaaattttct taatcatgac gcagtccttt aacttcattt tatcaggtaa 480  
 aaaaaagagc gaccgaagtc 500

<210> 38  
 <211> 300  
 <212> DNA  
 <213> Pig

<400> 38  
 acctgatcag gctctgcact gtgttcatca gcggagccga gatatttgac cgcccatgc 60  
 ataacggaaa ggcgtgggta aacccccggg cgcgttcctt tatcaagatg acgttcgaat 120  
 attcggcag gtgcagtttg tttattccag aaaggcgttg agcgcgtatg aatataattc 180  
 tgtgggattt gaagcatcct tttccctcct tcggtgaatg cgctgaaaac ggcttattcc 240  
 agccggttca gggtagcct gataatttgc attttaaata ccatttattg ggtacttttt 300

<210> 39  
 <211> 450  
 <212> DNA  
 <213> Pig

<400> 39  
 atccttttgg ggtctggcaa ttacgcaata aagaaggccc ccatgcgatt aaagtcaccg 60  
 gccactgtc gtctaatacat ggagaaattg tccatcagtg gggctctgat gggcagggga 120  
 ttgctctgcg ttcttggtgg gatgttagcg aaaacattgc cagtggatcat ttagtgcaag 180  
 tgctaccgga atattaccag ccagcgaacg tctgggccgt ttatgtttca aggctggcga 240  
 cgtcagcgaa agtgcgata acggtagagt ttttacgcca gtattttgcc gagcactacc 300  
 ggaatgtttc actgttgcat gcctgattta tgattcaatt atcgggttga tatcagttta 360  
 aaacctgatt ttctcctttc taagccgcta cagatttggg agcatattca cctttaatcg 420  
 cgcgatgatct aaagataatt gaagagggtta 450

<210> 40  
 <211> 450

<212> DNA  
<213> Pig

<400> 40  
aatgtactgg caaaaagcca atggcgaagc gtggggaacg ttacatgctc tgctggcgga 60  
tattaatagt caggggtcagg tgcagatggc gatgaacggc ggcattctatg atgaaagcta 120  
tgcgccgctc ggtttgtaca tcgaaaacgg tcagcagaag gtggcggttaa atctcgcttc 180  
agggtgaaggg aattttcttta tccgtccttg cggcgtgttt tatgtcgcgg gagataaagt 240  
cggcatcggt cgtctggatg ccttcaaaac cagtaaagag attcagtttg cgggtgcagtc 300  
agggccaatg ttgatgaaa acggtgtaat taatccgcgt attcatcca acgtcgcctc 360  
aagcaaaatt cgtaacggtg gttgggatta ataaacatgg gaacgccgtg tttttgttga 420  
gccagcaggc aacaaatttt tatgattttg 450

<210> 41  
<211> 400  
<212> DNA  
<213> Pig

<400> 41  
gacattaatc atttcaaaat caaagccccg gttttccatc gcccgtttgg tggcgtggca 60  
ctgaacgcaa tcgttacgag tgtaaatagt aatgcgcgat attcgtatct ccgtttaaaa 120  
tgaagatacg gcgcgatgat acgcgtcggg ttgtctctct gttgatacag agatactaga 180  
tgtagttgaa aaaagattca accacacaat atatagccca gtaggggtcg aaattaccct 240  
ggatatgagc gtgacggggt aggggggattt ttgtgattca ccaggcaaaa agaaaccccg 300  
aagacaggct tcggggtcaa agacgcgtat ttattatcat ttttgacta cgatttgccg 360  
atgcttaaca gtgcgccgat taaaatatct accgcagctg 400

<210> 42  
<211> 500  
<212> DNA  
<213> Pig

<400> 42  
gcaaaatcac gtccgcgacc tggcggtgtc gctgggccat attggcaaag gagctggatt 60  
gcgggtgcctg caaagtgcc tgaataatgc cattgtcctg taccgggaag aaacctttcg 120  
gaatgaacac ccacagcagc acgctaagca gcagcgtgct gagtgccacg ctttaaggta 180

gccacggatg attcagcact ttcgccagtc caccgaccata ggcgggcgatt atcctgtcga 240  
acattttttc cgaggcacgg gagaagcggg tctgttttac caacgactcc tggctgagca 300  
tccgcgcgca catcatcggt gtcagggtca gcgacaccac cgctgagatc aaaatcgcta 360  
ccgccagggg aatagcaa at tcgcggaaca gtcgcccgcg gatatcgccc ataaacagca 420  
gtgggatcaa caccgcaatc agtgagaagg tcagcgagat aatggtaaag ccgatttcac 480  
ctgcgccctt gagcgccgcc 500

<210> 43  
<211> 400  
<212> DNA  
<213> Pig

<400> 43  
agctatctac ggcaaaaggc acggtagtca atttcgttgt taaatacatc aagcgtttgg 60  
cgccgaaata ccatctgcca gatgccatct catttcgtag cgcactgcat aacggctacc 120  
ggatgcagta cgtcaaaccg gaactggggc cggaaggatt tagcttttct gcaatacacc 180  
ggcgggcacca ctgggtgtggc gaaaggcgcg atgctgactc accgcaatat gctggcggaac 240  
ctggaacagg ttaacgcgac ctatggtccg ctgttgcatc cgggcaaaga gctgggtggg 300  
acggcgctgc cgctgtatca cttttttgcc ctgaccatta actgcctgct gtttatcgaa 360  
ctgggtgggc agaacctgct tatcactaac ccgcgcgata 400

<210> 44  
<211> 450  
<212> DNA  
<213> Pig

<400> 44  
gattagcgcc agatgctcgc catcgaaaag ttgaatcaac cccagctcgc ggtaataagt 60  
gcgcgtacga acaaattcag tatccagggc tatcgccgga aaggcacgga cggtttcaca 120  
caaagaagcc agcgcatcgt ccgtggtaat catttggtta ttcaaattgt tttctcttta 180  
gtgggcgtca aaaaaaacgc cggattaacc ggcgtctgac gactgactta acgctcaggc 240  
tttattgtcc actttgccgc gcgcttcgtc acgtaattct cgtcgcaaaa tttttccgac 300  
gttagatttc ggtaactcat caccgaaact caccagcttc ggtactttgt atcccgtgag 360

ctgacggcgg caaaaagtca ccagtgactc ttcggtaagc gatggatctt ttttcactac 420  
gaagattttc accgcttcac cactggagcc 450

<210> 45  
<211> 750  
<212> DNA  
<213> Pig

<400> 45  
gagcagcccg cgtgatgaca ggcattgcgc cgcgtcggct ctctctctct ggtgcactga 60  
gtcacaggat ggcggcgggtg ggcgcgggtg tggaagcggc cctggaggggc tcgggagggga 120  
ggatgcgctc aagctggctc cccgtggggc tggcccggag tagcctccgt gagggcacccg 180  
tgtctgctcc cagagcccgc tccccggcct gcctgcctc ccttccctgc cccagttccc 240  
ccggagcccc tggatcccga tgggaggcgc ccctggggag aggggaccag ggagggggccc 300  
agagctctga ggccaccaga cctggccagg acccttcgtg ggaagaagag gtgggccccca 360  
aaggcaccta gagagagggga ggctctgctg gctggggggc cttccaggcg gggcttccag 420  
gcagggccag tgtcctgggg gctggaggga gtccctggct gctggggggc ggcaggagca 480  
cctggggcgt ctgggaagag agcgggagga gactggagcc aactgggggg acagaggagg 540  
gggtccaacc cagcgggtgg gttgggggtg ctggtggtgg aggccttgag aggctgtgct 600  
ggggggcaga gcggtgctg ggaggggaga aggggtcccc agggctcatg ggcccttcgc 660  
agcagtggca gttgggggtg gtggctgtct ctagggctgt accacgggtg gtgcctggag 720  
aaagaggtcc taccctagt ctttgctgca 750

<210> 46  
<211> 300  
<212> DNA  
<213> Pig

<220>  
<221> misc\_feature  
<222> (299)..(299)  
<223> Nonspecific nucleotide

<400> 46  
tggggacccc actccagccc cactgagtga cgcgcccccc tgtggtccca ccgccaacc 60  
tgctcacac cagaggggct gtggccacac cttgtccaca gcctgtccct gagaccacga 120

gcccccgggc tcagccccct cctcaccctt ggaccgagga gaagccccca cctgggctca 180  
 gctcttggag ctaaacttcc aggaagggtt tgggtgccctc ggggtcttaga gcatgggtggg 240  
 gaggggggatg ctgggtggggg cgcaagccct cccacattt cgcactcgac ccggtgggng 300

<210> 47  
 <211> 300  
 <212> DNA  
 <213> Pig

<400> 47  
 ccggctagaa gccacgagag cccccaggcc ccgcccgaag tctctcctgc agggattcgg 60  
 cagccctggg gccacagggc ctgagcagac cttgggggttc cgggtgtgact ccagccaggg 120  
 tccctactgt gtaggcacca gggcagagtc agccctggga ccatggccac agctgtctcc 180  
 gcctgagccg ggccccccgc ccaggctggg cccctcagt gcactgtccc aagccagctg 240  
 ctctccccac ctccaccttc tccatccagg tctgtcccca cggcctttgc tcaggcccag 300

<210> 48  
 <211> 500  
 <212> DNA  
 <213> Pig

<400> 48  
 ttgactggca ctagcacgag ctctgtaccc ggggatctgg gctcgggaga agggagaccc 60  
 cccaccggc aggccgaggg cgctgtcaca ccatgactct cagccttccc caccgacgg 120  
 acaagagtga ccctctcca agccccact caccaggac cgcacacccc gtgagtctctg 180  
 cgagtggggg cggctcaggg gccccgagtc ccaaaggagt ctgctggccc tgggggggag 240  
 gggaagcagc aggggtgtca cgggtctccc tgggtggcag gaccacaagc tcagcccgt 300  
 gcctcccaga gggcagccg acaccaacca gtccggggac cccacgtacc tcagctgctg 360  
 caggtgcccc tgctgtact ggtgccaatg gggccgctgg gtgctcccat ggacagctcg 420  
 cactcatcc cagccgccta ccccccttc ggggtccagt tccggccggc caccgcctg 480  
 ccagccctg gcctcctctc 500

<210> 49  
 <211> 500  
 <212> DNA

<213> Pig

<220>

<221> misc\_feature

<222> (500)..(500)

<223> Nonspecific nucleotide

<400> 49

gggggtgccc	caggctgctg	tgtaggtcgc	agacgcagct	tggatctggc	gtggctgtgg	60
ctgtggctgt	ggctgtggca	taggtcagcc	actgcgactc	cgatttgacc	cccagcccgg	120
caactcccac	atggcacagg	tcgagcaggg	aaaataaata	aatgaaataa	aaataggtga	180
agacagtgga	tttcatctct	tggggttgcg	gtaagctcta	cacaataggg	agtttaccat	240
tttacctggt	tcaagtggca	ctgagtcagc	tcacagtcct	gagggcccac	agatgccgtc	300
tgccctgggag	attgttcctc	tcaccacact	gcccctctgt	cccactaaa	tactcactgc	360
cctccccgtc	ccaagggcc	ctgccccacc	ctctgcttcc	tgtctctgaa	cttgctggcc	420
accagcgacc	gtctgggtgac	ctcactcttc	ggccccatth	gtcgcacacc	ccacctggcc	480
tctccccggc	atgggcagan					500

<210> 50

<211> 600

<212> DNA

<213> Pig

<400> 50

gggatatttg	ggggcatatt	tgggggggag	atccccacaa	ggcatttggg	gtttgtggtt	60
tggaatgccc	cggggcccga	tggagggggc	cggggaagaa	tctaagcctt	acttggggag	120
ggttgggccc	cggggccccg	ggccggaaat	gcccccaaga	cagaagtggt	acaaaatttc	180
tcaaaaggg	gaccttaat	gaaacgggtc	ccggttgga	agaggtcacc	aggggtggatt	240
ggtggcaccg	cagaatttac	gacattttgg	ctctcttcca	atggccggac	gcctggggat	300
aggcgcccc	gtggacggcg	gggtctcggg	tgggacgggc	ggtcaggggt	cgggtgacgct	360
tggcctctct	gaccgcctcc	agctccttgg	cgagcgtgcg	agcgcggcgg	gcgcgcagga	420
gggccgcgca	ggcccctgcg	caggcgttgg	gcggactgct	tccaggtgtc	atagcggaag	480
aacttgccca	cgggggtatct	ggggaagttg	tcctgagagg	ggaagggccc	gtcagggggg	540
ggcctggccc	cccagcccct	gtcccagaac	aacaaccttt	gcgggggtcct	cctgcctgcc	600

<210> 51  
 <211> 179  
 <212> DNA  
 <213> Pig

<400> 51  
 atcttcatat tcattgcagaa gacactctcc tgcctttcta tcttggggaa aaggacgatg 60  
 tcacttatgc aataaagccc acttgctggc cggggcttga cattattcct tcctgtctgg 120  
 ctctgcaccg tattgaaact gagttaatgg gcaaatttga tgaaggtaaa ctgcccacc 179

<210> 52  
 <211> 500  
 <212> DNA  
 <213> Pig

<400> 52  
 ctctgggctgc ttccaggggg ccttggggag ccatagaatg ctatggagca agagagtgc 60  
 atggtcagac gactttgggg gaaggtcttg gagaagaggg gtgactggcc actgtgataa 120  
 agagtggggc cttccttgag ataacacggt gggcagccga ggtggacctg tgcaggtgga 180  
 gaaggcctcc tgccgcggcc agtacgtggc tctgggctgc cggacacgag aaagcccacc 240  
 tccacggctg cctccaggcg gcccttcctc tcttcacacc gccggggccat gccaggtgc 300  
 aggtgccatc agaggggtgc caagagaagc tctgggctgg ggttggtcca ggtcccggaa 360  
 gccccgtgtc ccaggggcca cctgaggaag cgtgggcgca cagagactgt ccctcggtgc 420  
 tcagagaggg tcccgtcccc acggcaacga cgcccaaggc ggaggtggtc agaggtcttg 480  
 ggagggagga tggccgcgca 500

<210> 53  
 <211> 900  
 <212> DNA  
 <213> Pig

<400> 53  
 tgtgttgac ctgttgctgc ctgtcgactc tagaggatca atactcctta cataattaag 60  
 gagaacaaaa tggaacttaa aaaattgatg ggacatattt ctattatccc cgattacaga 120  
 caagcctgga aaatggaaca taagttatcg gatattctac tgttgactat ttgtgccgtt 180  
 atttctgggtg cagaaggctg ggaagatata gaggattttg gggaaacaca tcccgatatt 240

ttgaagcaat atggtgattt tgaaaatggt attcctgttc acgacaccat tgccagagtt	300
gtatcctgta tcagtcctgc aaaatttcac gagtgcctta ttaactggat gcgtgactgc	360
cattcttcag atgataaaga cgtcattgca attgatggaa aaacgctccg gcattcttat	420
gataagagtc gccgcagggg agcgattcat gtcattagtg cgtttctcaac aatgcacagt	480
ctggtcatcg gacagatcaa gacggatgag aaatctaata agattacagc tatcccagaa	540
cttcttaaca tgctggatat taaaggaaaa atcatcacia ctgatgcat gggttgccag	600
aaagatattg cagagaagat acaaaaacag ggaggtgatt atttattcgc tgtaaaagga	660
aaccaggggc ggctaaataa agcctttgag gaaaaatttc cgctgaaaga attaaataat	720
ccagcgcatg acagttacgc aatgagtga aagagtcacg gcagagaaga aatccgtctt	780
catattgttt gcgatgtccc tgatgaactt attgatttca cgtttgaata gaaagggtg	840
aagaaattat gcgtggcagt ctcttttcgg tcataatag cagaacaaaa gaaagagctc	900

<210> 54  
 <211> 450  
 <212> DNA  
 <213> Pig

<400> 54	
ccagccacca gctggaccct cccggagagg ggctgcctcc tctttcccgc ccagacgccc	60
cccagcaatc tgtggccaag agggagtgat accgaagatg gccacatggg ggcgccagcc	120
cacagggaac cccaggaagg cgctggaccg tcaggagtca gggctgctgt gcacccatgt	180
ggcctgggga ctttccacag cctggtggag atggccgggc acaccgctgc ctggggggaa	240
cgtgcacacg ggtggtacat gtggccggag cccagggcac agggtgaggg gagaaggag	300
catgcgggtg cagactcgga gcccgcgcgt gaggtgctgg gtcctcagga cacgctctgg	360
gagtggagga ccccatcca cgcctcacc cagtgtgtgc ccgctgctc ccccggaac	420
cctcacagac acgagggcac acccagcccc	450

<210> 55  
 <211> 1133  
 <212> DNA  
 <213> Pig

<400> 55



atggcgctca ttagaattcg acctcgggtac cttgggatct tttgacccct acctcacgcc	60
atctacaaca tttacctccg aatgaatgag agacaccaaa agcaaattca tagaagagaa	120
aaaaaggtaa cctggacttt aaaaatgtaa acttctgctc tttaaaaggc agtgctaattg	180
aagttcaaata acaaaccaca gaccataaga aaatacttgc aaatcttggt ctgacaaaga	240
ctagtgttca gaacatacga cgatcagggg gagggaaaacc agcaatccta taaaactgga	300
caaagaattg gggggaaaaa aaaccactt ggccaagaag ttggtaaata aggccatgaa	360
aacatgctca acatcatgag tcattagaaa aatgcaaatt aaaattataa tgagatacta	420
ctacacagct atttgaatgg ataaaaaatg ttttaaaaac tgattatacc cagggttggc	480
aagaacatga gaaacgagat tttcacacac gattggtgga aaacagaaaa tgggtccacc	540
actttggaaa agagctgggc acttcctca aaagttaaac atacatccag gacctcacac	600
aggctttcca ccacaggtgt ttattccaga gacatgaaag cgctcatcca cacaagact	660
cgtaaataaa ggtttatagc accgtttgtg gcccgaaactg agaaaacca aatgacctt	720
aaccagagaa tatctaaaca aaatatccat tcacattaat caccataag aaggaacggg	780
ctatggggac gggaaccgta ttgaagaggg tcaaaataca tacgcagcat caaagaagcc	840
tgcccaaagg acacacactg cagggttcca tggactgaaa ctcgagaagg tgaaaactcg	900
ccagcagtga cagagagcag gtccgagatc aacctgatgt ggaggaaagt gaacctcgt	960
gcgttgttgg caggactata aactggagca gcccctacgg acaacagtag cccgggctcc	1020
tctcctccat ctccctgggg agcctgagcc ttgagacgct ggggcaagtg cacggcatgc	1080
tgcctcacgt ggggccccgg tgaaaacacg tggcagctgg ggaaagaatc gta	1133

<210> 56  
 <211> 735  
 <212> DNA  
 <213> Pig

<400> 56	
tactgcctgt ctctatggac ttgactcctc tcgggacttc atgcgaggga tcttacagaa	60
tttgtccttt tgcactctggc ttgtttcact gagcatcgtg tccccaaggc ccatccatgt	120
tgcagcctgt gtcaggatct ccttcctttt caaggctgaa tagtactcca ctctgaggat	180
ggaccacgtt ttgattatcc atactagtaa atccatacta ataacttggt cactgaagcc	240

cacagcttat gctaccttcc gtggggtcct cctgtccctg tctctacgcc ttctgctata	300
gccccatccc ctctcatcca ggccacgcct cctgtcccct ggacactgtc ccagaagcca	360
actgccctct gactgctgct ctgcggtgac ggaggacaag gcaggctcag ggggccacgg	420
gctggggccc cagggtcccc catggctggg gccccttccct gattccagaa gtacagtggc	480
agcaccagct ttccagctgc cccaccttct gtccgcaggc tgctcgggtg ggggcagggtg	540
ggcagtgatg tcacctgctg taaccacct accgtcgctc atccctgtcc aggaggtcac	600
ggtgaccttg gcaaacattc tgaacaacac acacctccct ctgcttagag gccggggggc	660
tccccgggtg actgggggca caggctgacc ccagcctgtc tctgttctct gaaggacatg	720
ataagtactg caaca	735

<210> 57  
 <211> 500  
 <212> DNA  
 <213> Pig

<400> 57	
aggaagaaca ggaaacaacg gggttgagga gaagaaacgg gtgtctggca ggggcacgtg	60
ccaacgggtcc accgggtgct gccgcgctgc ggccctgggc cagagggggc agtccgccc	120
ctcggggcgc gccctgccgc ttgtgctggc tcgcggtgg gctctgcttg gctgggttac	180
agctgggtgc agccgcaggc tgtggtgggt gccgccgggt cagccagccc ggccccaccc	240
ggcccgtctc gccggcctgg cccgggcagc cctcctgcag tcgaggagtc gccctgacgg	300
gctgattggt ccacagcctc agatgcaaac cagccccacg tgcctggagc cagccagccc	360
gggacaccct ggtggaggca ggaaggcagc agcctggaga gccgcgccgg atgatgctgc	420
ggggaaaccg ggctcccgc gggggcgccc tggctctggc caggcttggc ttgaatgctg	480
acgtgagcgg tggccctata	500

<210> 58  
 <211> 500  
 <212> DNA  
 <213> Pig

<220>  
 <221> misc\_feature  
 <222> (112)..(133)  
 <223> Microsatellite PIGQTL1: (AT)11

<400> 58  
 tggcgttgca gtggtctctgg cggaggccgg cggctacagc tccgattgga cccctaggct 60  
 gggaacctcc ataagctgtg ggtgcagccc taaaaagcaa aaaaccccaa catatatata 120  
 tatatatata tataattatg gtaaaatata cataaaatag aatttacctt cttaataatt 180  
 ttcagtgcac aattcagtgg cactaagcac attcatgcgg ccgtgtcacc tgctccagaa 240  
 ctttccatct acccaaacgg actctccgcc ccatggaaca cgtccctgc ccctcccccg 300  
 gccctgcccc gccagctcct ccctgtgtct gtggatccgg ctctccagg gaccccgctgc 360  
 gtgggctcac agagtgtgtg tccctctgtg accgatcgtc gtgtccccga ggcccgttct 420  
 gtggcagctg cgttatgacc gactaccttc gaatgctcag tgactgccgt gcattggaca 480  
 cgcagtcgc tacccttttc 500

<210> 59  
 <211> 550  
 <212> DNA  
 <213> Pig

<400> 59  
 tgctttctgt gccccctcc agcttgggac cccagcaggg caaggggtgt atagggtta 60  
 aggaggcagg gggcgtctcc tcccgtggc tgcccagagc acccccagcc ccgcctgccc 120  
 ctgctccatc tccagcctgt ctttctctgt gccctccctg tcccgggcgg gccgcacact 180  
 ggcttccacc tccccacca actggcggcc cggctcttcc tgctgaggca ccccgaggtc 240  
 cccgctgctg gggaccagct ggcagggtggg tcccactgct ttctcagcgt gggctttgga 300  
 ggggggatct gcacatacca tcccttcagg ccccggtggg agcctgggga ccatccggga 360  
 cccctgtggg caggcccaga ggactgccag gaagagacc aggggaccag gcagctccca 420  
 ggctctcag cttcaggcca ggggagccca ccccagggtg gcagggaag ccaggcccc 480  
 aaccacaaa actgcccga gggaagtagg agggacagga ggaggggagg ccaggcccgg 540  
 gccgcccttg 550

<210> 60  
 <211> 819  
 <212> DNA  
 <213> Pig

<400> 60  
tgaggagcgc aggccccaggc ctgagtgtgc ccagcttaca cccctggcag ctctgtccct 60  
cctggcccta acccccatcc taccacagca gcaggggctc ccccggtggg gcctggtgag 120  
cgtctgactg gggtttggag tcaggtctgc tccaggtca gccccatcc ccaagggcgc 180  
cctgcagcac tgctgccac cccctagcgc cccagacct tcgccccctcc agcctggatg 240  
taccacgga ccctgaaaag tggggctgag caggtgccct ggctggagtc cccctgactt 300  
ggggctggcc aggctgccct ggaggggctg tgggggcaca gcctgcccc ggggcccgt 360  
gggcactggc tctggagctg acgacaggca ggccctctct tcctggcggg gccacacct 420  
gccctgggggt ttggggccaa ggcgggcacg ccccatgtca ggcgggggcg aaccaggtaa 480  
ttacagcctg gcagcccgt cccagacct ccagccccgg agggcccca cccaggtgt 540  
gccaccaaga cctggcatcc agggcccaa gcaggtcaag ggcagctgct acagattctt 600  
ttaagttgag acagaatcga cacatgacaa gttcctgggt ttaggtactt cgctgccggg 660  
gccgccagtc agtttagtga cccagcacac cccacacagg tacaattgct cttctcaaaa 720  
gagggcccctg agagagcgcc tgtcttggct caggggtaat gagcccaatg ggtatccatg 780  
aggttgccgg ttccatcccc ggcctgcggc cgttgggtta 819

<210> 61  
<211> 500  
<212> DNA  
<213> Pig

<400> 61  
ggctcaggaa gcgcaggggc agcgtgtggg gcgacgggaa ccatgggggt ctgtcttccc 60  
gcctctcttc aagcccaccg ccctgctgcc cacctccgac tctgcagca gcatgccggc 120  
tagagcccct gtgcaccag ctggtggcct ctggctaagg gcagtgtggt ctgtggacgc 180  
gtgtcccctc cccagcagcc caaggggtccc atctgccagg ctggtggctg aggtctgccc 240  
tgtgtggtcc ttgcaaaaac cccgccctct cctgcccctt gaggcgtgag ggagacgcgg 300  
gctgggcgga tgccctcggg cacagccgcc cgcgggtggc cctgtcgag gagggggctc 360  
cgacgtgccc tgacggccct ggccggggcg agaggggtgag gccacctct ggccacgtcc 420  
accagctgc cacgccgct agccagtggc ccggggccaa gtcagcagag ccaggcttcc 480

gacaagcaga ggctgtaggc

500

<210> 62  
<211> 700  
<212> DNA  
<213> Pig

<400> 62  
gatgaggaag ccgctgctcg tgctgctcgt cttcttggcc ttggcctcgt gctgctatgc 60  
tgcttaccgc cccagtgaga ctctgtgcgg cggggagctg gtggacaccc tccagtttgt 120  
ctgcggggac cgcggcttct acttcagtaa gtagctcagc ggggcacggg ggcggggcgg 180  
acacagcagg tgctccatcg gtgctgcccc ggtacctgtg cgggtccttc gggatggatg 240  
gtgtggggga cggggggcgg ggggcggcca agggaggacc tctcctccga gggctctgaga 300  
cttcagaccg ggggcgccct ggccgtgcgc attgattggc acctgccatg tgcctggctg 360  
gggctcacac cccctgacgt tctgcagcg tgactcgaaa cgggaaaccg aaggacggg 420  
tggcacgggg tggggaggca gaccgtgagt ggcaggcgtg cgaggggttc tttcgggcgg 480  
gggtggcccag gcaggcccca caggatgaca gcctgtcccc tctgctcct ccttgacctg 540  
cccacagcca gggctgcagg cactgacatt caccatggt attgtggtgc cttgacctt 600  
tggcagtggg cattgggttc atggactgtt tggattgaaa agtgggaata agatggggtt 660  
tgaaaaaccc aattaagaaa taaaaggcg cctgtgggc 700

<210> 63  
<211> 300  
<212> DNA  
<213> Pig

<400> 63  
tttgaaaaat tttgagtcag tgcagaatc gcctctattc cgcattcagg ctctcctgtt 60  
ctcaccttgc cttagtgcgg atcttctata accaccacag tgacgttttc aaggtaacttt 120  
attgaataat aagaaaaaag tgcacacaat catgtagtta actttctgtg ctctttgccca 180  
gtttgaaggg accctctttt tttccttttt agggcttcgc cgacggaagt tcccgggcta 240  
ggggttgagt cagagctgca gctgctggcc tacagcacag ctcttggcgg cgatggatcc 300

<210> 64  
<211> 450

<212> DNA

<213> Pig

<400> 64

tcttgggcca caggctgcag cagctcacct gggggctggg gtctcgctct gcggatggac 60  
ccatgaaggc cggagccagg tgggggccga gacggcaggg caaagggctct gcacacacag 120  
cgtccccccg acccggttc tctgggttct tgggggggttg gcgaggcttc tctcagtctg 180  
ggtttcttgg ggaactttca agaactggga agtcttccag aaagttgggg tgaggggagg 240  
taccaccaaa gtgtgtctcc tgtccccatc cccacccccg ctgtccatcg gcgagacccc 300  
ggaccgccgt ctccctgccg aggtgtgggg tccccccctc tgccggccag gctgggcagg 360  
ggtgagcgcc ccctgtcttg cactcgggac tcagcctggg gaaggcgggc cccaggaggt 420  
cctggcctgg acggcagtga ccttccaccg 450

<210> 65

<211> 500

<212> DNA

<213> Pig

<400> 65

tgtgcatcca acccagtg ccacgggggg tgaccctcgg ccggtcagcc gcccgctct 60  
cccacggaac cgggccttgg cctgaggcag aaggaccag gactccatcc ctgccccgga 120  
ctctgccgga ggggtgcggtc tgcacagaga ccctctgggg gtgaggccgg tcggggctgg 180  
ggttgagatg ggatggtcag ggcggcccc gcgggcctgc aggaggctgg gtgaaggagg 240  
gggcccagct cagacgcccc caaacctagc ttgggagagc tgcagccccg cccgtcaat 300  
cgcgacagcc tgcccacaga aggcattcaa atgagagaca aatatttggg cttgaagact 360  
atacccagcc acgtctcttt gggagcccaa gctgctccca ggccttcatt tgggtattaa 420  
ttggttttcg tttagagatt tgcattgctta tcaatggcca ctgggaggct gggcctggat 480  
gcgggtcccag gctttgtatg 500

<210> 66

<211> 661

<212> DNA

<213> Pig

<400> 66

tcccacgacc tgcccctcca gggccacatc tggcgacacc gtcgcaagag ttggaccggc 60

ctggtgtggc cacagcctca ggccttgtct ggccgcccag gccggctcca ggctccaagg	120
agctcctgcc tgccctccgg aaccccagca ccccgggccc gcttccccac cagacctgtt	180
tttccaggtc aaggtcacag ctaatttggg cttaaactgg acaaggaggc cttatctgga	240
gcaggctccc ggcccttttg cctctgccct ggtggggagg ccttcccaga ggctgtgtgt	300
tggcgctgac cgtgcagccc tgagcttgaa cccggataag gagggacccc acctgggctg	360
gagccagaga gccctcgttc ccagctccg cagggttctc acagtcccgc cctgccctg	420
gggaccctgg acgtccccag caggtgaaag gtccagatgc cctctgacta gaggtcctc	480
cgtgtcaga catgctccct tcccgaccg aggacgagac ctcagcagcc ctgctgggcc	540
tggggtgccg accccaaggc gtctctgagt gtgttctaata ggggagccgt ggggcctcaa	600
cagtgggggt ggcacttgga ggggagcctc cccacagctg cccaagatg ggccctggac	660
t	661

<210> 67  
 <211> 500  
 <212> DNA  
 <213> Pig

<400> 67	
tttgttggat gaatgaaatc atgagaaagt gattggaccg ccccgttcgt ccagctgctt	60
gccagctgct ttgtaaagat gacctctcac cttctcagag gcctggccgg cccgaggtgg	120
cagtcagctg agatgccatg cttgtttggc acgtgggagg cccctgtcca cggcgtgggt	180
gcctcttgtg tctaatacagg gtcaggggga gcagcaggtg cagggcacat gtggggccgg	240
ggccgatgtc tggggagggc gggaggaggg ggtgtgcgga ggccgttgtg ggggtgcagg	300
ggacagacc cagcgagacc ctccctggcc aggcaccagg acaggtgatg gggggccgcc	360
tccggggcgt gtgacagaag cctctcagag gaggcctcc cacggtctct ggaccatcaa	420
gggaccgggg gcgctgggcc tgggggtcac acccagctgg ccggccagcc cgggtgggggt	480
cggaggcccg ggcagttcac	500

<210> 68  
 <211> 550  
 <212> DNA  
 <213> Pig

<400> 68  
 gggcaggagg gggccggggc tgggtgcggag ggtggagggtg gtgcaggagg gtgtgaggca 60  
 gggctcactg agcgtgcgag gctggctgtg ccctagagtg gttagcacgt gccccacccc 120  
 tccagtgtcg ctctgttcac ctgtgcctgg ctcacagggtg tggaaactga gactcgggtg 180  
 ttgcatgagc ttccaggatg agaatcagca ggcttcccag gcagggtgtg gtccggggct 240  
 ctgggctctt accaaggagg ggacacccag ggacagccct gcttgggggt gtcgggctgg 300  
 ccaggctggg tggctccttc tgtggctggc agcccttggc agtcaccccc ttaccctcaa 360  
 ctgccccctca gctgagacac gacctccctg cagagccctg tccaccaga cactcactcg 420  
 cctcctccag gaagccttcc agggctgcct cgcctgggtc tcagcaggag acagagagag 480  
 aggggtgggccc caggagcaga ggcaggcagc cagaggggaa gcccaggggc cctcactcac 540  
 ccctggggccc 550

<210> 69  
 <211> 500  
 <212> DNA  
 <213> Pig

<400> 69  
 tttgcattca gctcgtaccc gggatccttc ccggggggtc tgggggtggg ggaatggggg 60  
 tcagaggcag ctgtcatctg cctgtcctac ctgtctcac aggttgcccc tggagccctg 120  
 gcctcctcct aggggcacat cagggttttg gggaggccca gccaccgtc ccacctcaa 180  
 gaccacagct gggagcctgc cccccaagcc tagacctagt ggggtcctg ccagccaggc 240  
 cccaccttc atgctgccac ccaccaaggt gggacagtgc agccaggaca tccagcttct 300  
 ggagctgccc gaggctcagc acaggctggt accctaggga gcaggtcacc cagggcggcc 360  
 tggcgaggcc tgcggggacg gggggtaggg tgggcagcaa aagaacctct gagctgggccc 420  
 gggcggggtc ggtgagggcc cggggccgag ggctgtgtgc gtggccctg agcccggtgca 480  
 gacgcagacc ctgggtgggt 500

<210> 70  
 <211> 550  
 <212> DNA  
 <213> Pig



<400> 70  
 tgtgctgctg tggtgtggt gtaggccgcc agctgcagct ctgattcgga ctccctagcct 60  
 gcgaacctcc atatgctgct ctaaaaagac aaacataaaa taaaatgggt gcgctgttaa 120  
 tttgaacact ctgcctcctc cagagacgag gccgaaacag gcctctctga aggtcccacc 180  
 tggcagggag gaggaggcca gcccctgtgg gggcagagag aagcccgatg tcccagaca 240  
 cacacgcaca gggaccgtgg ccccggtgc cagccccgcg gggggagggc aaggccagag 300  
 actcccagca gccacagga ccttggtggc cacaggacac aaacacaggt gacggtgggt 360  
 gaggcctggc ctttcccccc ctgggcacga gcacaggaca cacaagagcc ccagcgtgct 420  
 gaccgccacg ccaaggagcc tggatgaagc tggacaccga gagtccacac tgtgtgatta 480  
 ggctgacgtg aagtttaaga acaagcgggt ggctcagcgc ttgaaggcca gaacaaggcc 540  
 gggagggcag 550

<210> 71  
 <211> 1300  
 <212> DNA  
 <213> Pig

<400> 71  
 atgtcaggat agtaacctgg ggtgctgcag tgacaatgcc agatccttaa cactgtgcc 60  
 acaagggaac tccttgacct agaatcctat acccactgca aatatatttc aaaaaaggta 120  
 aagtcctgag cagaaaagca aaaatgggat aattcatttc tggaagacct tccttgtaa 180  
 aggaagtttt ttggacgtga tgaaggtaga aactcggagg cacacaaaga aagaaagaaa 240  
 gaaagagcac tggaaacgga gcaaataaag gtaaaaataa agttcatctc tttctcattt 300  
 ttttaattgct ccaaaagata gctgacctct aaagtaaaaa atagtggaaa ttagcatat 360  
 gtctctagcg taatttaaag tataacttat agcaatgata gcccaaataa aggaggaatt 420  
 gagaatatac agttgctgtg tccccattgt ggctcagcag taatgaacct ggctaataatc 480  
 catgaggatg caggttcaat ccctggcctc actcagtggg ttaaaggatc cagggttgca 540  
 gtgagatgtg acgtatgtca cagacgtggc tcggatctgg catttctgtg actgtggctg 600  
 tgggttaggc cagcatctgc acctccgatt tgacccttag cctgggaacc accatatgct 660  
 gctgggtgtg ccctaacaga cacaaaataa aataaaaaata aaagagagag agaataatacc 720  
 attgtaaatt tctcacatg acacaaagag caatgtgata ttatttggtg tatggtgatt 780

gattcaagat gtatatcata atattgattc aagatgtata ttttcctttt ctaaaaaaga	840
gattttatata ataaggcaag agtgaaaata aagtggaaatg ctaaagaata gttaatccaa	900
aagaaggcag aaaatgggga aaagacatat aacagatgga acaaataaaa aagagctaata	960
gagattgtaa aatttaatat aaacatacag ataattccat taaattttaaa cactctcaac	1020
acattgatta aaagaaattg tcaaattgaa taaacaaagc aagaccaaac tagatgcaga	1080
ctatgaaaaa cccacttcat ataaagacat gggtaggttt agagcagaat gatggggaaa	1140
ccatgtcacg caaacatttg tcaaaataaa gctgggtgtgg ctgtattcat ctgagacaca	1200
gcagacttca gaacaagaaa cactgcaaag gatgaaagag atactgcata atgataaagg	1260
gatcaatttt ccaagtgcag gctccaaaca acagagggtt	1300

<210> 72  
 <211> 500  
 <212> DNA  
 <213> Pig

<400> 72	
atgacctcat actgaatcga gctcggtatc aggggatctc tcagctgggg gggaggggcaa	60
tggggcattt gtctgaggat gccccagggc agggccattg gctggtttgg tgcccatgcc	120
ccccccacac cccggcagtg cccctgtctg agcctgggac cccctctggg agttagggat	180
tgggggtggg aaccaggctt tgcagtaatt ccagccccca gggcccttcc ctccccgccc	240
tcaggacccc cagccccgcc ccacacagtc tccactgtga cagcctcacc ccttgggtca	300
agtccctgtcc tctccggccc ccgctgggca gtggagccag ctaggtgaga ggcacaggcc	360
actagggcgg tgggcactgc tgaggacaga ggggcctggg tggccttggg cgaggcccag	420
cgacgctgag acagtgagcc aggtccagg ctttcccagg gaggtccct gaatgtccac	480
ttcttgtgac atcgggtgac	500

<210> 73  
 <211> 550  
 <212> DNA  
 <213> Pig

<400> 73	
aagtccatta gggaagggat ttgtgcaaac acagagacag gtgcagggtt gggccagctg	60

ctgggctggg ggctcctcaa gggccccgta aaccctccc tgccagccgc ctgccgcaa	120
ggtctgctgt ccaccccgge cgggctgctg tgttcccggc gtgtgtcctg cgaacccgac	180
tcccgttcac cctgagcac tgctggagg cggctgccc aggcgggacg ggccctcagg	240
gctgggctgg ctcttggcct gtgtttcatt tctgagcagg tccttctcag tggggggggc	300
cttgggtgaa gcaggcatgt gcaccactgg ggcctgtcc ccagtgggca tcctgggcgc	360
ttgtctggcc cccaaacccc caggccgtgt gcatcatacc ttcacctga gcccagccg	420
aaccccgac atgtgctggg ggaccctggg cacaggggtg agggagcagt ggccttgggtg	480
gaagcccagc cttggcacct ggggaggggg tgcactctggc atgctctgct gtaaccaagc	540
ccagggcagg	550

<210> 74  
 <211> 950  
 <212> DNA  
 <213> Pig

<400> 74	
gacgtgcagt agccatgacc tctacggccc cactgacca gccctgtgcc ttgtcccag	60
accgaccct aagcaatagg atgcagcaga agtgacagaa cggcctccgc gatgaggctg	120
cagagggctc tggctctgac tcaggccct catccctcgc tctcctggag cagggccagg	180
taggggcccc ccagagacgc cctagaggag gtgacgggca gccagccgc ccaggggaag	240
gcctggggac accaggaac agaacggcac aggtcctctg cacagtctcc caggagcccc	300
ctgggtggcac agaaatcctg accggcccag tggagggggc tggggcgggg ctcggggagg	360
agggactggg tgaggccgtc tgactcctgg ctgagcgccg catacttgct gcctgccac	420
gatgccgggc caggccttcc gcacggaccc aggtcacat tcgccctaca tgccactgtg	480
tgggagtttg ggatggtgtg cccgctgggc cgggggtca gggcacgctt ccagaggag	540
cgggttccag aaggcccagg tggagaggcg ataggaggc tccaggggc ttcccaggcc	600
acctgcgagg accctcctgg ggggaaggga gcggaggag acagccgggt cccttaggcc	660
aaggctgagt tgtgaccgca gggagaggag agaaggagca cccacagcag ggcaggggct	720
gcgggaggct gtgctgggtg gccgggtggt gggctctggg gccaggaccg tgggaggcct	780
cgagggggga gcaggcacgg gaggggcccc tggacggcag agtccctgct ccagctgccg	840

ccccgacccc aggtccacct tcatttcaca gcctggcccc cggccgctct gaccggccct 900  
gcccatgcag gtgtagcggg gcagtgaggg ccaggctccg gccgtcccaa 950

<210> 75  
<211> 450  
<212> DNA  
<213> Pig

<400> 75  
gcaggcctgg cagcagggaa atgatccaga aagtgccacc tcagcccca gccatctgcc 60  
accacactgg aggcctcag gggccgggcg ccggggggca ggcgctataa agccggccgg 120  
gccagccgc cccagccct ctgggaccag ctgcgttccc aggcgcccgg caagcaggtc 180  
tgtccccctg ggctcccgtc agctgggtct gggctgtcct gctggggcca gggcatctcg 240  
gcaggaggac gtgggctcct ctctcggagc ccttgggggg tgaggctggg gggggtgca 300  
ggtgcccctg ggctggcctc aacgccgcc gggtccgcag gtcctcacc cccgccatgg 360  
gcctgtgga cgcgcctcct gcccaggtt gggcccttgc tggccctct ggagcacccc 420  
gcccccggg cccaaagcct ttcatgaaca 450

<210> 76  
<211> 1363  
<212> DNA  
<213> Pig

<220>  
<221> misc\_feature  
<222> (206)..(206)  
<223> Nonspecific nucleotide

<400> 76  
cctccagctg gggccggcag ggcaccgtgc ccctcagggg acaccacggg gggccacagt 60  
ggcctctcct gctccagget ctgtcccgcc ctggggcccc ctgggcccgc cggccatggc 120  
cagggcaaac tcccagtgcg gctgcccgtc tgggcaaaga ggccgccagg ccccgctgg 180  
tcttagcagg cactggcgga tgccgntaac taaccatttc ttccgcagga gtccgaatct 240  
gctctgacca cgggccctaa aaatcgctcc tggcccgagc aggatccccg aacagcgggg 300  
ctgcctcctg ctctccttgc cgggcccggc ctgggcaggc acgtgccctc gtcgtcccca 360  
gtctgtcaac cgtcccgctg ttacgatccc cagagtccca cgcgcgggca gctctttcca 420

caccccgcac ggccccggag ctgcctgggc acccagatcg cccctgacgc ctttgctcct	480
aattctgctg aaatacacat aacgtctcct tgaacgtttg tccattttca cggggacaat	540
tctgtggccg taggtacact ccccttgggg cgcagccatc gcaccatccg cttccaggag	600
gtcccgctcgt cccagatgga cactgtcccc actgatccct aattccctgt cccccccagc	660
cctgcccttc ctgtctctgt ggccttggcg cctccaggga gccctgtgc gtgggatcac	720
aaaacgtgtg tccctttgcg tccggtgtgt gtctctgagc atccggagct tggggtgctt	780
ccacgctgcg cctgtgtcag gacgtccttc ccttttgcgg ctgcgcgatg ctccccgtgg	840
ggctgcccc aactgcgcgt gttcgctcat ccatccacta aggctgagtt acttttggcg	900
gttgtgaata ctgctgtgtg aacacgggcg tgcaaatacc tgctggaggc catgctctta	960
ggcctctcgg ggggcacacc cagagcggat atgctcaata aggtaattct gtgttttagct	1020
ttttggggaa ccatcaggct ggtctccaga gtgacggagc atgcgtcgca ttcacaggaa	1080
tgggtgctga ggctttgagg tctccaccac tcgcttccta ttttctgtgc gtcacagccg	1140
tcggaacggc tgggtggtgc ctctgtgtgg cttcaatgtg ctttttcttt tcttggctat	1200
gaggttgagc gttttttatg tacttgctgg ccattcgcag ggttttttggg gtttcttttc	1260
ttttttgcct ttggggacgg cgcccagagc gtatagaagt tccctggctg gggactgaat	1320
cagagctgca gctgccagcc tagcccacag ccgcagcaac gca	1363

<210> 77  
 <211> 500  
 <212> DNA  
 <213> Pig

<400> 77	
tcatgccatc gccaccgccc cccaccccga cgtttcaaac accagaacca cccctcgggc	60
ggcagagaga ggacnggaag gagagacagc ctgggtccaa ggctcgcgc ggtcctgtgt	120
ctccgagcga catttctttc tgtttccctc ctccgcggtc caagtttcac ccatcagagg	180
cgcattgttt tcatcatctg aaaaaaaaaat ctctgtctct taataaaaaca caagaaaaag	240
tagccttcga aagaaagcac atgaatgata tgtgctggcg acagtgtctg cggcctctga	300
gccgtggtgg gaggtgggag ccagcggagc ccctgaccga tcacgtgacc cacgtctctc	360
ctgcacagct ggctgcacct gcacgcggtg acacagggac ccagcctcct gccagcaggt	420

cacccccacc cgtccgtctc ctgtggaagg ggcagcgttg ctttctgagg gtgggctgct 480  
ctgaggggcg tcctttggcc 500

<210> 78  
<211> 626  
<212> DNA  
<213> Pig

<400> 78  
gccatgggct gggcggttc acgcggcttg ccggcctgcc tggaagtccc acaggaccaa 60  
ggggagggca cgtcagcaca ggggccccgg gcacggacgg tgccccagc cgccccggcc 120  
cccgccctcc agacaggacg cccggtcacc ttgcggggac agccagcctc gtggcctcga 180  
gcagaagaag tgagagtggg gtgcacaggg gccccccggg gaaggagagg ggacagcggg 240  
ggtgagcggg tgccggcgctg ctccggacca gcccctggcc tcttggcgcc tcctccccg 300  
tccttaaacc gggcccagcc tcttggcct cgacccaagg ctgtttggaa aataggtgga 360  
ccgtggccct gacccgaagg ccagcgggga cccgagtgcg gtccccaatg gatcagcagg 420  
cgccctggga gcctgcggcc ccgggacccg gagacacagg tgggaatggg aggaggagga 480  
ggaagacggg aggagaggag tgaggaccag cagaaaccac gccctcctct cttcccgctc 540  
tcgccctcgc ctccgacagc tccgactcgg ctgcaaggaa aaggccccag ccagccccg 600  
cgccaccggg gggggggggg gggggg 626

<210> 79  
<211> 500  
<212> DNA  
<213> Pig

<400> 79  
tactcgggtt tgttaccact gagccacaaa gggagctcct aaaaataata attttcttaa 60  
agccaatgac atggagagca gttaggttg aggctggtg gtggtggggc cgcggcaggc 120  
gccctgaagg tcctgagtgg cacccttggc cgggggaggt ggggtgggca ggggtgttga 180  
gaaggggcag ggccctcgtg gggcaggaag gaagagccag tggtcccgag tcccctgacc 240  
ttgctgcctt gagcctggtt ctccccaaaa ttctgtctgt gtcccttcac ttcacggaag 300  
cttggggccc gttgccaggg agacagatgg gctggtgaca cccaaaatga gccaccagga 360

ggggggcact gacttttagcc agccgggtcac atcaagaagc aaacaggccc cccgctgctg 420  
 taaaggcagc ttggggctgg ggtccgggag cccccctgg gctggggaaa gggggtcctc 480  
 tcaggcccc ggaggatg 500

<210> 80  
 <211> 427  
 <212> DNA  
 <213> Pig

<400> 80  
 tctattcgcc gtggccggaa gaggctaacc gtacattgac cgggcatctg gcgatgtatc 60  
 acttctctcc aaccgaaact tcccggcaaa acttgctgcg tgaaaacggt gcgtagacc 120  
 gaatcttcat taccggtaat acagtcattg atgcactggt atgggtgctg gaccagggtga 180  
 tgagcagcga caagctgctg tcagaactgg cggcaaatta cccgtttatc gaccccgata 240  
 aaaagatgat tctgggtgacc ggtcacaggc gtgagagttt cggctcgtggc tttgaagaaa 300  
 tctgccacgc gctggcagac atcgccacca cgcaccagga catccagatt gtctatccgg 360  
 tgcattctcaa cccgaacgtc agagaaccgg tcaatcgcat tctggggcat gtgaaaaatg 420  
 tcattct 427

<210> 81  
 <211> 650  
 <212> DNA  
 <213> Pig

<400> 81  
 ggcgttgccg tgagctgtgg tgcgggtcac agatggggct cagatcccgc gtggctgtgg 60  
 ctctggccta ggccggtggc tgcagctccg attcgacccc tggcctggga gcctccatat 120  
 gctgcgggag cagccctaaa aaaaaaaaaa aaaaaaagga agaaaagaga agaaagaaaa 180  
 gaaaagacaa aagtcaaaag gagctcccct gagcgatgtc tgtctacgag caggctccctg 240  
 ggagcctgag gcagggtgag cctggacccc tgagggccac tccagactca gtgctctcac 300  
 tggccaaggt ctttggggac cggctggggg cgcgcgcagg ctaaggagga ggtcagagga 360  
 ggggcttcag gctgcagggc cagcggcagc tctgggcccg gggcgggggg gagatggcct 420  
 gagggccttg cgggggctgg aggggtgggg gcttcctgga gtgggaagac ggggaagccag 480  
 gtcagaggag aggagcgagg gctgaagctc ctggaaggcg ctggctaccc ccagctggcc 540

cgccccgctg ccacattcaa cagccacccg gcctgtggtc ctggcagggc cctggcagaa 600  
aagccccaag ggccccagcc tggccctctg ggccctaaaga gccaaagcccc 650

<210> 82  
<211> 550  
<212> DNA  
<213> Pig

<400> 82  
ttaacccacg gagcaaggct ggggatcgaa cctgtaacct cgtggctcct cgtcggattc 60  
gttaaccact gcgccacgac ggggaccccc cagggctggc gtttccctct gtgtgcacac 120  
agtggacctg agccaaccag cagggccttc accaccacgg cgcaagagtc ggcagcaaga 180  
gagcagtgtc tcatggctca ctttctcccc cttccccgga gtggtgacaa aaccccgccg 240  
ccaccggact cggttagaca aggcgggtgc cagtgcctcc gtctgtcacc cgcacggcac 300  
ggcgtctctc tttctttctc ggggctccac cacgtgtcct cagtttccgc atgagagtac 360  
cgcggtctggc ggggtggtgg ctctggggtc gggggccgtg agggcagggc tgggctgggg 420  
gaggcaggtc ttggccatt acgcgggggg cagactccac atcacacgct ctctgtgcct 480  
cttggtctgc tgacaccatg gacttcaaac aggaacagcc gtggaggcat tgcagcccag 540  
ggccccgggtt 550

<210> 83  
<211> 550  
<212> DNA  
<213> Pig

<400> 83  
tgacacctcc aggcaggagg gtgcaggctg gggctccagg taatggtgtg ctggcctgtg 60  
gggcgtgggc tcagctctta ggatggtggg ctgggcgccg acccagcaag gacaggggtga 120  
tggcaggctg tgggctcagc aaatgagtgc ccaggttgtg ggggtgggca cttggggctc 180  
aggggaagct catcagcttg gagagggacg ggggagggag ggggccttgg ccagctggcc 240  
cagatgcctg gatgtgagca ctcacgtgcc ccggggtcca cctccccctc agtgccatct 300  
gggcaggagg ctccgatgcc tgtccctggg acccgtgtc ctgaaatgag gttcacttgg 360  
tgccttcccc agagatgtc ggtccggaag ctgacgaggc aggagtgcac aagggtcttg 420



ggaaatggag cagagtgcgg ctggggcaca gaggctgccc ccagcctggg aagatgggga	480
gctttgcagg ggtaccccg cagcttgtgg ggccctggat acccaaggggt gtgaagaggc	540
tgaagagcga	550

<210> 84  
 <211> 984  
 <212> DNA  
 <213> Pig

<220>  
 <221> misc\_feature  
 <222> (679)..(679)  
 <223> Nonspecific nucleotide

<400> 84	
ctgagcccag ctatgtagat tagaccccg tccgtcccaa attcttctca aagctgtccc	60
gagatgagag atgaggtttt cgtgtcctgt gctctcctcg cttccctgg gatgtgccct	120
agggtgggag agggtgtgtc ccagggtca gcaggcggtc ccatcttccc gagacgggag	180
agateccctc cttctcggcg cctgtcccca cggcccccac agacaccccc cccccggca	240
tggcacccat gcacctgcca tcgtgccag taggggatgg gtttggcgag actggagatg	300
gctgtagcca gtgagacatg ccctgccag tagcctgacc ccctgggtgt gctctgtgag	360
atctggggac cccagcaca cctagggatc atctttgcca gcctcctggg gagcctctca	420
gaaatggggg ccccagaag gctggcaaag gtgatgggga gcgtgggaag tctggcggtt	480
ggcgggggtg gtggggggca gtgcgggctg ggtgggggggt gctccgggggt cggaagtgggt	540
ccagcaaggt tttggacaca aagtcaggag gaaggagtga cgaggagact tgcagaatta	600
caggtagaat caggaaccca catcgacgcc aattgatcta tccccccctt tgattgtttt	660
ctcctggggc ttttttccnt tttttttttt tttttttttt ttaatccctc cttagctttt	720
tacgcgctca acaccaaatt aaacgtactc cccacccac gtaacagggg ggcggtgacc	780
cgaaggacga ggagcacacg aagccaccat ccgtcacctt ggcggcacca gccgctgtcc	840
tgccctccgc ccatttatcg cccttgaatt gatttttgtt ttgctctgtc cctgtcgctt	900
gggtagagtg gaaaagggaa cctctgtggg ggtgccagcc actgggcccc ccaaagattt	960
caggggaatg aaacggctgc cgcc	984

<210> 85  
 <211> 550  
 <212> DNA  
 <213> Pig

<400> 85  
 tgcccctgac aaccctgccc tgtagccac actcgcgact aataaggcga gaggtcagcg 60  
 ggcagcccca cggggagaaa gtgcctccgt gccccccacc cctgggtctg atggcccagc 120  
 ctggcacccc aaggtggcct cggccttcct acctccaagg tccaggcgca tgtccaagca 180  
 ccagcagaag cttctccagg gttggtgcct gctcagggca gaaagcaggg gtgaggctcc 240  
 ccaaagggcc actggcacca atgccccag gcagccccag cgaaggggac agcccacccc 300  
 cagcccgggg acgcaggcct gaggggacat ggggaacca gagcagggcc aaggggagca 360  
 gagccccctc tccgggactt gaaatctttc ccggggggcc caggagctg gggctctgag 420  
 agggcacttt caaaatacgg cccaccccca aattgccacg tgggccacag agcaaggagt 480  
 cgctgccaaa gtggcctggc ttcagcgag gaagtcccc tcctggggcc tcccctccta 540  
 taggcacagg 550

<210> 86  
 <211> 500  
 <212> DNA  
 <213> Pig

<400> 86  
 tgagccaggg cctggcccag ctaagcccct ggagccctcc cggcctgttt cctgcctccc 60  
 atgctggcgg agctcggctt actgagcggg ggccaggcca gtgtgcgtgt ggaggtagat 120  
 tccactcagc tggaggttga ggtgggcagg gggccgcaga ccctcaggcc agctctggcc 180  
 ggccaggctc ctgaagctcc ccgggtggc ctccccgtcc ctgcctctgg ccttgtcctg 240  
 gcccttgctt gacaagcttc tgtgggtctg cctgcaggag agacactggc tccccgctc 300  
 tcggatgagg acggggcttt tctgcacaag tcctgcccc gaatgtttgg ggcgccagca 360  
 gctgagccca gcacgtctcc cctgcccct ggctggacac gaatcccggc atcgaggcgg 420  
 gaagggggat ggagggatgg ggcctacca ccctgctcc ccaccagaa tagctgggcg 480  
 gccccatgg gaggccgccc 500

<210> 87  
 <211> 913  
 <212> DNA  
 <213> Pig

<400> 87  
 ctgttttcac gtcttctgag gacacacca gaagaggggc tgcaggcgcc catggtgact 60  
 ccatgtgttc actgctgagg cctctgcaga ccgtctcccg cagcagccgc acccgtttcc 120  
 atgccaccaa cagcgtgcga ggccgcactg tccccacggc tgtgcaactg ttttgaatct 180  
 gagttatata agcaacagac gctccttcaa acacactcac gtgcacacgt gcgcacaggg 240  
 gcacagacac acacacggag taataggcct cccccccctc cctgagccca gagggggcct 300  
 gggggccctgg agcctgtgct ttagggcctt ttaggaaagc tgggtgcctcc cagagggggc 360  
 gccccgagcg ttggcttccc aagtccccac caaccctcga cagactcaaa cgttggtttc 420  
 tttcgtgctt ttgccaagg gatgggccc aggtggccct gcctgaggtt tcagcccagc 480  
 gccccaggca ccttttctct ccggtcccc ggccacttca tgggacagcg ggccttcccc 540  
 cacgttgctc cctgggttgt cgtgcttttc gtaatgagac ggaggcaggt gcacctgtcc 600  
 tggggtgaat tctcttctgc aggaactcgc ttccccggcg cctgggtctgt ctgttcctcg 660  
 gttgttgga cctctcgtca ccagaaaggg tggctctgac gtcgcccttt cctccgtgg 720  
 cttttgcagt ctgggtcttg tcggggaacc tgccccaaag aggggagtga cccccacga 780  
 gggagacgta gctcctgtgg cgacagcacc gggggccccc agattcatgg ggttcacgt 840  
 cacagtcgca tgacgctgcc tttggacgag ggcagctcaa gggaagcttg tttcctgcc 900  
 cgagccacag gca 913

<210> 88  
 <211> 650  
 <212> DNA  
 <213> Pig

<400> 88  
 tccacacctg tggagccgct gcctcgtgta tgccctctgc ccagctgatg gtcagggtgcc 60  
 cagacttggg gctcagtcca aacagggggc cacagggtgt gcacctgggc aagggagcct 120  
 gtgcgcaggg cctcagggtgt ccagggtctg ctgggaccga agcgcaactgg gtccctggact 180  
 ccgggcttcc ccaggggctg ctgggggcca cctggaaatg aagccccacc tgggtcatag 240

ggtccacgtg agggccctga ggccaccaag ccaccaaaaca actcagttaa gggagggggag	300
cttgggggctg ctaagctcca agcgggaagc ggccgcactc agcactgcct ctctgccagc	360
cagccgcccc gcttgctgac gtcccaacca ggccagggac cctgtcccac agatgctggg	420
cccttccagt ctctgctccc tggaggcgct gggcactgtg tgggcacaca gcccgcaccc	480
gcctgtaagg aagggaagc ccccatcctc aaaaaagccg tgggcaggtg ggccatgatg	540
gtcctccgag gcaggtcctc ctgggacccc ttgctcctc gggctcgccc aggagccgcc	600
aggctctgcc tggattaact ctgccccgca tgtcattttc aaactggctt	650

<210> 89  
 <211> 700  
 <212> DNA  
 <213> Pig

<400> 89	
tggggccctt tggggccgga gcggccagtc tgctgggccc gggagcaggg ggtctctgtc	60
cgcagggagg gggcctggtc tcaggggagg agaggaggca ggtctcacct gaaaggatct	120
gccttctcct caggcctctg ggatgcctgg gcagagaaac cagaaggaaa ggcccaactt	180
gctggctggt ggggatgggg ccgggggtcg ctcccggcac accccccca aacccacct	240
tagtggccaa agtgggtgtc atgatggcca ctgacctcac gggggcgcag gagacaacaa	300
aatttcagcc actcttgggg gaaggacact tgtggcctga gtcttagggg ctgagtttcg	360
ggggggaccc ccagctctcc cccagtatg agacaccctg cccactcctc ccagctgctc	420
cccaaaccga gtgcttctgg acgggcatct cccgctgcc cctgcagccg ctgtcctctg	480
accatgtccc ctccccacct cccctctgca ggccagggc tccagggagc agagccgagg	540
ccccacccta gactgagctg gggaccgaga cccaagtcg ccaccgggtc tctgcgttag	600
agaggggggt ccggggggca ccctggggcg gactggggg gcgggaagga gagccctggg	660
ccgttctggg aaaggtctgg gagggaggga ggggttttgc	700

<210> 90  
 <211> 1400  
 <212> DNA  
 <213> Pig

<400> 90	
gcacaccgag agaacagagg gaggggtcct taccagtctc aggggtttttt tggggatttc	60

tttgaacttg ccctattggt ttogaggctt ctgttctctc caatcccccc ttctgaaccc	120
ccccaaaaat gggttcagcc cccaccccag ccagaggaaa ccaattgggg gattgggggg	180
aggcgggggc agcaaaaagcc ttggggcccc agccccctg gctttggcct ctggcctgcc	240
aggtaggggg agggacgcgg tgacctccgg gggcctggcc acggactctg cccccacccc	300
cagggcagac gtgcacagga ggggagaggc tccgaggaat gaggccatca aagggaacagg	360
tgaggccacg agccgtggga cctggaagtg tttagggcct gggggacgag gctgcggcct	420
gcgggctccg tggtcaggag gccctctgcc cactgagcag ctcccaccac tggcacacga	480
gcctctctgg ggtccggctg gtctccggca ggggtgggct ctgaacgtcc agctccgcag	540
acaaatcaga ttcccccgag ccctgagaaa gccccctccc ccagcccgtc tccccacctg	600
tcgggtggaca gagtgacccc tgctgacccc ctgcccgggc tcccgagga gatgtgagag	660
agtaagaggc ggtacaggac ggccggggcg gcccgggcga ggtgcaggctg tgtgggtgtg	720
aggctgggca caggctggca cagcctccct ggcccagtc cttgggcacc tctgggcacc	780
tcgggtgtgcc tgcctcctga agggatccac cctccagcca cctcctctcg ggccagcccc	840
cacccccacc ccgagctaca gatgcctgcg cattcgcccc aagtgtcctg gaccctggag	900
ccaggcagcc caccgctca gcctggccag acccagcggt gcccttcacg ccctcctccc	960
tcccgcgggg tcctcgcgct cgtctcctca ggttgggaagc cccttcccac ctgccatctt	1020
gcctgcgccc aggatacacg gctcaactca aggcctcact cctcgccctc tccaaggctc	1080
tgteccaggcc cctctctgac ctggcaccac ctgccgcctc ctggcagccc cagcaaaccc	1140
cctgccacag tccacgacag tcctcttctg gctctgcccc caggatgctt ctagaactgg	1200
gggggggggtc cttccagccc acgcagcatc cactgggccc tgggctccct cccaggtgc	1260
ccctcagagc ttgcagctgg tgcagacggc tctgctccga acccatgctc cctgcgcctt	1320
tggacctggt gagatgttgc aggtcatttg gctgcaccca aaagagtggc ccctcagggt	1380
ccccccctgag cccctccatc	1400

<210> 91  
 <211> 391  
 <212> DNA  
 <213> Pig

<400> 91  
gtactgtagg gcctcattcg aatagcctac taggtcacag ctgatccaca ccttaggccca 60  
tcacaacttc ccagaggttag tgccgctcct gtcgttgaac aagacggttag tgactgctgt 120  
gagagctcag atctggtggg tcactgaccg agtgtggaac cctgggggaa ggctgtgggg 180  
tgtccccggc tgggtggcca tgtcatgtgc ccccttctat cccttgagc aggctgggttc 240  
acteggtctt agagcccaaa gcccagctg ctctgccaac ccccaagcc tgagcctcat 300  
cagaccacc accccatcgc catggctacg caggacacac cgctctccac cccaccagc 360  
cgccccacct ccccgagggt ccaaagcttg a 391

<210> 92  
<211> 1464  
<212> DNA  
<213> Pig

<400> 92  
tccaggacct gatgcagcag ccacgtcgcg agggccctcc cagcaggccc cttgttgacc 60  
agcgctaggg aaggggacca gggagatgct gagaacgggg ccttccgagg gggcagggtgg 120  
gactgactgt gaccaaacac tccccacccc cctctccgc tccagagggt gccagcctgg 180  
aagctggcaa agtccaatcc acagggtggc tcacgtgggg aggctgggtg cccccacctg 240  
gtggggcccc aagctgcctc tgggcggggg gggggctgct ccagcaggg tcccatccag 300  
cttctccctg gggagactca cagttctggg agaagggtcc tgactgcacc gcagcgcccg 360  
ccccctccc agactcacc aagttctctc tctgcatcgg tgactggtct ccgcatttgc 420  
ccaggctggg catctgccca gaggatacgt ccaaaggcag ggcaaagccg ggcccgctcc 480  
ccggagctcc ccacaggcgc tgagggtctg gctggatctc gggggggtgg aggggaggac 540  
tcagaagggtg cagcggggtg gagcgaggct gagccaaggt gcacgcgagg gccagagaag 600  
gccgaggcgg gcaggaggag agagcgccag cctggagggg ggtgggtgcc ctgggcagggt 660  
ctggggctca agaagaagag agtgtgtgtg cagggggctg tccaagctgc ccgggagggt 720  
gcctgcccac ctccaggag caaagcaggg aggctgcagc tggcccggcc ggccgctctc 780  
caggaccacg cgtggcccag gcctcaacgc tctcccaca gccaggaga cccagggcac 840  
cgggtccatt taccggggc tccgggtccg tttgcctgcg ccctgggatg gactgtgggg 900  
gcggggcgct gtctggggag gagggaggtg tctgaggctg gacaccttga aggcagggtga 960

gagtgacagg tccgtgcgca ggagccttcg gctctggatt ctggccctga gcgaggggct 1020  
 ggctggaaac tgggcccggg ctgccgcagg agagtgtgca gggagaggag acgggggttg 1080  
 gccccggagg tgccgggggtg gtgccctgga gtgcggctga gcgggaagtg ggtgttggcg 1140  
 tctggagacg gggggtcgtg ggcttgggat ggtgacaaga cccccagggt ggaggcggcc 1200  
 gcagaggagg cagagaagcc agggcccagc cccacggcgg gaggcctggg agtcaggagg 1260  
 gaccagcaga gccctgggct cagtgtcacc ggtcctggca cctcgccgac ggatgtcctg 1320  
 gccgtgcagt ggttgtcccc tcacctgag ccctgagaac catgcaggat gctggtgtca 1380  
 cagcaggaga gggccagggc ctggggagga gtcttactgg aaggccttct cttccgttt 1440  
 gcagcaggcg ggaatgactg gggg 1464

<210> 93  
 <211> 694  
 <212> DNA  
 <213> Pig

<400> 93  
 tggagccagg gcacggcaga gcggtcccg ggcctgcgt gctgaccgg gggatgggcg 60  
 gacctggggg tgggctgtga gccaggcat agggaccccg acttgggcac ggccagggtg 120  
 ggccgggcaa gggggaacaa ggacgctggc ctccaagggc cccacgtggg cacagaggaa 180  
 gagccgaccc aggttgtggg cgcattggaac cccccactct gggggccagg aggccgaacg 240  
 tccaagggc tgaggctggg aggggaagagt ccctttgggg gtcagtcagt gtcccttgtg 300  
 ggtgcccccc tgccactggc ggcacctctg accccaactc cttgcgggtg gacggtggat 360  
 ggatttcctg cagcctttct tctggaatag tctctgccat cctcggggaa gcagtgattg 420  
 ctctgcccaa gtccaggccc cgccctgcaa ggtgcctccc accccaatga gccccggac 480  
 agttcgaggg cttctcacgc tactgagggg tatgaacagc tgtccccctc ggaaagtggg 540  
 ggacaggccc ctgccactcc atcctcgga cgcccggtct agtcagcact tgtctccctg 600  
 ccttgtgccc ccctgacctt ttttgaggac catcaaaacc tcagcctctg cccagaggag 660  
 tcaagcccc cgccccag ccccagacc agca 694

<210> 94  
 <211> 900

<212> DNA  
<213> Pig

<400> 94

ccagccccat	cccccggtg	gtccccacc	acacagagcc	cccgtttccc	aggggacagc	60
acagcctgcc	cccaggtctt	acataaagtc	acctttctcag	agctcctgtc	gcggctcagg	120
ggaatgaatc	tgaccagcat	ccatgaggac	acaggtttga	tcccaggccc	cgctcagcag	180
gttaaggatc	tggcggttgc	gtgagctgtg	gtggagggtcg	caagacgtgg	ctcagatctg	240
gtgtggctgt	gactgagggtg	gcggccagca	gctgcagctc	tgattggacc	cctagcctgg	300
gaacctccat	atgccgcggg	tgagccctg	aaaggacaaa	aataaataaa	taaataaaaag	360
aagtaaacac	acctttctta	gccataacca	cctgcctagg	ggcggaggggc	caggaagcgg	420
cacccccgc	cccaggctgc	ccgtgcgccc	cgggcaggcg	gctcagcctg	ctttttgtct	480
gtgatgtgag	ccgccccagc	cccacatgga	ggggctgggc	tgcgagtaa	ctgctttaac	540
tgacgggagc	ttcgaccagc	aattcaccag	cgggcatgca	gccgggaagg	gaagttattc	600
gtgtgtagct	attaggcgcc	ggagtgaggg	tgtgcctcgc	cctggggcca	cccctggggg	660
gaggcatcac	aggggttttg	aacacctgcc	catgaacacg	gggcaaaagc	cagccaaggg	720
ggcagggtgcc	tgaggctggg	aaccaacctg	tgtctctgaa	atccggggaa	tgcccactgc	780
aggeatgttc	aaaggggtcaa	gaccggggct	ctgcctgaga	aggactggcg	aaggccaact	840
acaaaagcgc	accctctgt	gcaaaccccc	aaccaatgga	acaaaactcc	agagggggcca	900

<210> 95  
<211> 550  
<212> DNA  
<213> Pig

<400> 95

agtctgggct	gtgtccatgg	ggttgccaag	gtgccaggca	gagaccttgg	ggacaaaggt	60
cctgtgagca	gaaggacatg	gccacgtccc	ctgctcagca	ggtgcccagg	ctggggtctg	120
atgccctcgc	tgggggtggg	gcgggttgag	gggccaggcc	cagacacct	tcgtccctgc	180
cggagttgtt	tgcccttctg	ttcctggaag	gccccctgc	aggtacagga	ggcccctggg	240
gctgacgctg	caccttctga	cacctgtggt	cttggggatg	ggacaggaca	gggagacccc	300
ggggctggac	ggagcgggta	agacagagag	ttgactctgt	cctcgagtct	gtgcagggct	360



gtccccggct tgggcttcgt ctgcagggcc ttctcgggtca ggggtggcctc aaggtgacga 420  
agacctgggtc ctctgggagtc tgcaggcgca aaagttggag cccaccccc cggggagggg 480  
gcgccaagga caggagggcc cagggaagtc tggggcctgc aaggccgtcc gggctgggga 540  
aggccaaggt 550

<210> 96  
<211> 1200  
<212> DNA  
<213> Pig

<220>  
<221> misc\_feature  
<222> (1074)..(1144)  
<223> microsatellite PIGQTL2: (GT)8 GCACGCGTGTGCGTGTGTAC (GT)17

<400> 96  
gtttgctctc agcaggcaag ggcctccgag gccttaatag ccataatga cagcgccgc 60  
tcctggcatg gggccccgcc tggcatggg cagggcaggg cagagcaagc agcatgcagc 120  
ttctaccttc ttctgacct cgtggccct tccgaggcct caggggggtcc ccgagtggg 180  
acccagcccc tggtctctct ctccagagcc aggccaagg ctgggagtgg ccagagatg 240  
agggtgcccc agcagggcac tgccttggcg tccccatccc tggcgctca gggccgtact 300  
gtccaaaacc aaaagaaagc agtcagcaaa acttctccca gcaagctggg gtcaaagtc 360  
gcttccgagg cgtgatcagg gtggcctttg ctactgtcac cgtgtgccct gggagaggca 420  
cagggaacaca gacacacacc tccgagaacc tggggcttcc agggcgtcag gctgcctggg 480  
ccatcccggg ccctgtgggt ccaggatct gccgggaccg tgaggcctgc gtcccacct 540  
ctgcctggga caggccccac agagctcaca gccaggggac cggggacagg gccccgctg 600  
ggccacctgc ctccagctc accagcctg ggccccaggc ctgtgcctgc gacacctga 660  
gtctcaggac gggcgcgga caaagccgcc cggccccctcc ccgggtggg aggagaccg 720  
cgtggccctg acgtgtgggc ctgtcagagc tgaaatgtca cagcaattag ccctaacgag 780  
gccgagggag ggagcggcgg ggaggccggc ggaggggatc cagagccga gggcccgag 840  
ctggccaccc caccggtcga ttccaggcac tcagggataa ttgggtgttt agaagtcagg 900  
cggcagcaga gagcgggcca ggcgggtgt gccccccctc ccaccgccc ttaacagggtg 960

cccgaacacg caggtctggg gagatgctga ggtcgccaag ggcacccctg gccgtgccgc 1020  
 ggggtgctatg ctggttcggc accatgggag ctgcacctgc agctgtattg gtctgtgtgt 1080  
 gtgtgtgtgt gcaacgcgtgt gcgtgtgtac gtgtgtgtgt gtgtgtgtgt gtgtgtgtgt 1140  
 gtgtacgtgg gggggggggg caagcccgtg cgtgtgggtg acagtagaca tttagaaggt 1200

<210> 97  
 <211> 600  
 <212> DNA  
 <213> Pig

<400> 97  
 ggggaccagg gccagccct ccagctcca cgcatacctg ctaggagctt gcaacctgcg 60  
 agagctttgt ggacccctg ccgggtgacc cctgaagctg gcagctctcc ttggctctgc 120  
 agcggctctc tacactacc cctctccagc ggccctcggc ccagacatca cccaccgca 180  
 agggaagcag caagcatcca ccagctggc ctttttccc cagcctgtga ccggccccgc 240  
 gccccctcac acctctgcg tccaagacc ctctctggct gggccctggg gctgcccttg 300  
 ccgtgcacat ctgggggtcca taccaccca acaggcccca cttttctgtc tcccagtgtc 360  
 cccctcagct gccctgatgg gccacacct ggcttctctg ctgccccct tgaccgcaaa 420  
 aagactgggg tccaggacc cctgccccat gactgcctg gaagacctca agcctctcct 480  
 ctcaatcctg accctttaag gctcttgcca cggagaaagc ggctgggggt gggggagggt 540  
 gtgggtccca aagcagcttg catacttctc ctgactggga gctcattcct ccacagcgtg 600

<210> 98  
 <211> 1350  
 <212> DNA  
 <213> Pig

<400> 98  
 cccgccttat ttttaaattt ccgaaaacaa aaaccacacc tctcccgtcc ccgaaattat 60  
 tttggtatag tcttattcaa agaagtctg ccaactgaagc ccaactgtcc tgtcccgggc 120  
 tgctttggcc aagggccctg acgggcccag ggtggctcat tcccgcattc ccgcagaggc 180  
 cgccttcaca tcccatgcgg gagcctggct tccggcacc cggctgtgcc tcgctgtggc 240  
 catggactgc tttcgcagaa gcataggggc cacaacatgg gacagcctcg ctctgctcgc 300  
 tgtggttccg ctgaacctct cagctggaca tctgggcagc aagcacccca gctttgcttc 360

aggctctggt tccaggctgg gccctcctcg gccctgcccg ctgggtgccca agcagggctg	420
gtccggctgt gccccgggt ctatagaagc ctctgcaggg cttcctacag ccaggctggg	480
attcgggggc tgccggggac tgaggcccc tctgagtctg accccccat ccttcctcc	540
cacacagccc ccgcccccg cttctgcttc agtgaggccc caccctgcct cactcgctga	600
catttccaga acaggggggt ccaggaagcc ctgagcctgc aggggactca gtgaccagcc	660
gcatttgaat tttccctcct tctgatctct ggagacagct ctggctcagc ctggctcgag	720
tgccctgagc tggggaccag gacagacctg cagatggagg tctgagcctg ggcagggcag	780
ggcccaaggc tcaggagaaa attgcagggt tgagatcaat gaccggagcc tggatggggc	840
cgccctggcc agggcagctt tctccctgca gctccctgcc actgtcccc ccaactctgg	900
gctcctgctc tggaccagct tgtgtgttcc cctcctccca gccgagccac cctccccat	960
tctgcccccc ccaatccaac accctatcgt gggaaccagt ggagctgaaa gaaggacccc	1020
ccaagggccc ccagccgct gtaatccttg ggggcctctg ccaggtgcc aggtctcggg	1080
caggaggggc cgcgggcaca gccgtggcag atgcgcccc caagcctggg ctcgaggag	1140
ccccgcccc actgacattt ccaggccgcc cgctgcagac cgggctggcc gtgatattta	1200
gacagggctt atttgccgtg actggttttt gatgactttg gggcccagga tgagctcagc	1260
cgagcccgcg ttggcccacc ttggtctcag cttgggtttg ataataaac gcgttcaact	1320
gaaccgctga cgctgcgtg ggccgaggcc	1350

<210> 99  
 <211> 1354  
 <212> DNA  
 <213> Pig

<400> 99	
gcttgcagta gttcatcaga ttggacgact cataaatgtc aagacatcta aagattggtg	60
catccaatca tttcccacca ggttgTTTTT ttgtagatgt caagaagctg acccaaaaac	120
tcacgtggaa atgcacgtca actgggagag ttgaaacaat ttctaaaaag aagaaggacg	180
tcgtgggagg actcttcgcg ctctttggtt tcgcttcaact ttatattatt agttactgat	240
tttctaaaa gctgcagtag tccagacagt gggcctctat gaaggagg gctcagagat	300
ggttgggaca gaatagaaag ccagaaaacg gacccccgca aatgtggtca attgagtttg	360

ggcaaggatg tgaaagcggg tcagtggaga agagtctttt caagaaatct ctggtcctgg	420
atccactgct catccaggcc caagagtga cttggcgcac atttctcaca gtgtatacaa	480
aaactgactc aaaataattc acataccgtc gtgtagcgta tgaagccatg aaacatccag	540
aagaaaatct cggtaacctc agggcatctg gggcctccac cctcagcacc actggccttg	600
gggccagata cttacgtgtt ctctgtgca ctgtgggacg tgcagccaaa cccaacaag	660
gtgaccatca gaaatgtctc cagacgtcgc caaataactg ccagagagca caggagcccc	720
tcactgagaa ccacaggggtg gggcagagag atctcagaca tgacacgatt aggggaaaac	780
aatctgacac actggctttg ttaaatttaa aacttttccc ctgtaaaagg caatggtaag	840
acattaagag gcgaagtggc agactgggag aaaatatttg caaatcatgt atcagatacg	900
aagaagatgc aggaaatcct caaagttcag tcacaagaaa acccaattca aaaaccagca	960
gagcagacat acgatggcaa ataaccacga gaaagtcagc acccgctgtc cctgggggga	1020
cgcgagtcaa agccaggagg acaccaggat atgcccactg ccaaggctac ggataacggg	1080
aagcaagaga cacagacaga aaggatgctt cggtgctggg gaggggtggg tggggcgggg	1140
gggtcccccc tggagcagga tgtgaaggca cttggggggg gctctgcact cctggggggc	1200
tttggcacag ggggagggcc cgggaaggct ctaggggcac ggagaggggt gccaggcttc	1260
cttaccacgc ccaggcagac caggccctgt catgaagcct gacgtgcagc agcaagagca	1320
acatgctaca gacatgtgtc tgtgtgtgtg tgtg	1354

<210> 100  
 <211> 1000  
 <212> DNA  
 <213> Pig

<400> 100	
ggttctcagg cgcacggggc agaggctgag ggtccgaggg gctttgggtg ctggaaagcc	60
tgagtttgaa tcccagctcg gtttcttaaa gctgtgtctc cacggccaag gaatggggcc	120
tctctgggaa aggtctgggg tgaggctggc gggacctgcc agccccggag ggcatctgac	180
cagacagctt ctcaagctca cagggttca tggcaggatg gggaaggctg tgggtggggag	240
tggggagcac tcgacacct gtccaggcct cttgagtcac ggtggcctct gaaaaggggt	300
tctctgtgtc caatgagcaa gtctttgtcc ggggcaggat tactaagtcc aagggtgtct	360

gcccctccgt ggggcacaga gcaggggccc cagatcacgt ggctgtaact gccaggttgc 420  
 aaagcctgcc accatgtccc actgggttct ccagttacct tgggaggtgc aggggtgggt 480  
 gatggggaaa ctgaggcaga gagctggcaa aagagtgccg gcagggactg cgggcgccag 540  
 acccagctaa ccgacctca cacggagctg cttctacttt gcagcctgga cgtgggaaaa 600  
 ggttacccca cagcagcgtg tgcaggcacg ctggtatgtc tgtgtactta tgcatatgtt 660  
 ctacgtgcat gcacgtgagt gtgctgtgtg cattgtgcct gtgtgtgtgt gcatgtgtgt 720  
 gtgcactcat gtgtctatac gtgtgtgtag tgaatgcttg tgcattgtga tttgcatgtg 780  
 tatgtttgta cgtgtgcagt gaatgcatgt gtgtgcagtg gcggcatgtg cgtgtgtgcg 840  
 catgtgtctg tttataacctg tgtgtagtga atgcatgtgc atgtgtgtgt ttacatgtgc 900  
 acgtgagaat gtgcactcgt gcatgtttgc atgtgagttt catgtacaca tgcttttaac 960  
 gtgtgcacgt gtgcacatgt gttctgtgt cccttgcacg 1000

<210> 101  
 <211> 1500  
 <212> DNA  
 <213> Pig

<400> 101  
 cgtataaata tattaatata gaataaaata gattgataat atagataaac taaaccatt 60  
 atcaataaccg ggtggcccca gcaaaggata ctagccagtt tatcaagggtg ctaagtcagc 120  
 acatagaatg gccacaaacg aaaacctgta ctgcctatgt ccactctaatt ggagtatgcc 180  
 actgacatca gtggtagggtg agctgagtcc atctgggctc ccagttcggg cccggcttgt 240  
 cccccaacgg aggttccttc cagggttccc caaacccaac cgggccccca ggtctccctg 300  
 tcttgactcg tttctggagt cttctggggc tctgcagtcc tcccttggtg gggcttctgt 360  
 cccctgccc ctggccttgc gggctcggcc ctgcctggg tcccgggcct gcgggctcac 420  
 cctccttctt tccctggaag agagggagcc aggtcgggcc gggccaggag ggaatgcgcc 480  
 tgactctgct ccagatggac aggtcgggac atgcagtggc ctgccttgg gctgctgagc 540  
 caagagcagg acgggttctt tctggaatgt ggggccagcc aggttcagcg tgtgggtggg 600  
 cagccgccag catctgtcag ggccgtgca ggcgcgggga atgacctga cttctgcttg 660  
 gcaccagct ctggaacagc cccctgcgga gcctccgcc agagctgggc cagaggggtcc 720

cctgtgccgg	ggaccccagc	agggcccctc	cctgactctc	caaccacact	gcctgggagg	780
agtggccccc	tggcctccgt	ggatctcttg	gtcggggctc	agccggcttg	acagcctggg	840
aacagccaat	gcacatcccc	aggcctggcc	acacccttcc	accgggagcg	ggcggatctg	900
catttcgcca	ggctctgcgg	gcagctctga	gagccccggg	tctcggagcc	cagccgtggc	960
cgttgtagcg	cctgggggct	gtggacagcg	tgtcctcatt	gccctccga	gtccggccc	1020
aggccccctc	ccacctgctc	gccagagacc	ctctccccac	caaccacact	tcctgctggt	1080
ctgcaagcgg	gacacacact	ccggtttcag	gacctttgca	cgtgccgctt	cctctgcaga	1140
gaaatgcctg	gagcagatgt	ttgtccgcac	ggctgctccg	cgaggcctac	cgagagcccc	1200
tcacctaaac	ggccgggcct	cagcagcccc	gggccctgtc	cccaccgccc	agtggtggg	1260
ttctcctgtg	ccagtgtggg	catctctgta	agatacctgt	ttatctgctc	atcgtctggt	1320
ctccccaga	aggtagagca	gggcccggca	cagccgtcct	cgggggtggc	actcgcctt	1380
ggggctcagc	ctccatgcag	ggagggacgc	ctggtgacac	gagagccccg	tgtgagtgtg	1440
ccgggcccgc	agcctgcctt	aggtcacagc	caaagccggc	attaaccacc	aggccctcga	1500

<210> 102  
 <211> 600  
 <212> DNA  
 <213> Pig

<400> 102	
tctagaatac	ctggccctcc agggacgtgt cctgtagctg cggctttcag ggcaaagtgt 60
aattaaacat	ccccaggctt ccctccagt tggcacaggg caccacatg aggagcagcc 120
tctgggtgcc	aaagggccca ctggtgccag gcgctgggct gagtgcaccc ccgcatgctt 180
ccgcccact	cacctgctgg cccacccct gaccacagca cctgtgggaa cactaggcct 240
ggcagccaca	cgctgctctc actggaggcc agtgccaggc agcctgcttg gctacgctag 300
cagatgcccg	ctgcctctg cccctgcccc tagcccatgc aggagcccag ggtggggcac 360
aggaaggacg	attggggccc caggtcaggc acatccaggc cacagccgtg gccacacgaa 420
ggcggccctg	agggggcggt ggggggcaga ccctgcccc ccgctgccgc cccagctcca 480
ggcattaatt	cccagggacc tgttgactg ggtggccgcc agcctgcccc cttgccttcc 540
aaggcctcta	aatgccctt cttttcgtaa actaggactt accaagctca gcgagccctc 600

<210> 103  
<211> 1867  
<212> DNA  
<213> Pig

<400> 103

agtatatcgg	gtgagactgg	ggaccggtct	gccgggaagc	cccaccataa	aggccacgtt	60
gggccacagt	cggggccacg	tgagtgtggg	cgggtccgcg	ggtctgctct	tggaacacca	120
ggatctctaa	gaggtaccag	ccgaggccaa	gttcacgtga	gcaagtgagc	aatgactga	180
atgagagcgt	gagcgaatga	gtgaggggtg	agtccgtcca	ccacgcagcc	taggctcagc	240
caaccgctgt	ccccgcgtct	ccactggtga	ccagaacgga	aagagtgggg	aaagagtggg	300
tgtctcccac	aaccagttcc	ccaaccccc	tggacgcccc	accctccag	gggtgccggg	360
cctggcctgt	gggccccagt	ctggaggctc	tggcaccttc	ctcatccgtt	ctcccagcac	420
cccaggttcg	tgctgagccc	tcctggccca	caggcctcgg	ggacaaagag	ggccacctgg	480
aggctcaggg	agcctcacct	gcctcgtggt	cctggcggag	gcggtctctg	acatgtgata	540
gaccggcctg	ggctcagcag	ctcctgctgg	aagatgtcag	ggacagcctg	ggccactctc	600
ccaccaggag	aacttattcc	tcggtggggg	ccccccgggg	aagggatggg	atcccagcgg	660
ggaccccaga	gcgtccagca	cacggacctg	tccctccagc	ccctgcccc	cacggatgct	720
cacagctcag	cctcgaacac	gcacctgttg	gactttgcct	cctgaggctg	tcttctcagc	780
cgacgcgggc	ctccgctgca	tggtctggaa	gccagtgagg	actcgggtgg	gacagggaac	840
aggggctctt	ggagtggggg	gccgggggag	ccccgagggg	gctgcttggg	cctttgatgg	900
ctgagtgggc	tgaagtcagg	cagggtcccc	cagggtcccc	tgaccccccc	cacctcaaaa	960
aatccagagc	atcctttgct	ttgggtctgg	tgaggctctc	tgaggtcaga	ccctgcgtgg	1020
ctggggccagt	ggggctggag	caggaagaaa	gcaggacagc	ccccgcccct	ggcccagact	1080
ccccaaacc	agcaggagac	acctgaaacg	ggatggaacc	atcctgaaaa	gagccacctc	1140
ctcctcctta	tgcctcagct	gccggggtct	gggggcccgc	cccaggcccc	agatgtccgg	1200
gctgctcccg	tctcacatcc	aggggtttct	gggcccagga	ctctgtcccc	ccaagcatgc	1260
agaggggtcca	ggctgggggc	ttcatgcctg	cccgtgtgca	tggtggggaa	ggaaggggac	1320
agtctggaga	ccccccgccc	tccccatgcg	tggcgccggg	ggacaaagcc	ggctgggggc	1380

tcaggtttgg gttcagagca aacgttgatc tgacctggtt ctgagatgct cggcccgatg	1440
ctgcgttgtc cgctcgcatc ttctctgttt ctctgggagg cgctcgctgc gctgtggctt	1500
ccggccagcc ccacggaggg acgcagggtg gctggcgggg tctggggggc cctgcccga	1560
ccagaacgtc tggctcaggt ttttgtcctc gtgacctatc actaaggggc accctctgac	1620
ccggagccct gtctccgagg tgggaatttg gggctgtccc tggcgtcata ggacctggtt	1680
gggggcatcc agggctgtgt catgcccctc cccagaagac tctgggggct gcgggagggt	1740
ttccccagct tcggggcagc ctggggaggg cgggaaggcg tggaggcctt gcctgtccca	1800
gggagcatgg cttcgtgca gactggggcc ccgcacaccc agccaccact ggccgtctgg	1860
aagcact	1867

<210> 104  
 <211> 650  
 <212> DNA  
 <213> Pig

<400> 104	
gttgaggatt cctcggcaat ttctcgtca ctggcgctcc aatcgctcg atgggcttct	60
cctccagata cagctgcaga tcctggggcg gcacaccgtt gagcgtcacc tcgtagtgc	120
gattgcactc gttgtcaatg gacatccagg ccatgccgac ggcattgtgga ttctgtgcat	180
ccgtgtgctc ctgtcgcttc agcagaatgg gttccgcca gtcccagca tcggccactg	240
gacggggcac taggcggcca cggatcaggc tcgtctcatg ctcggtggcc acattaacgc	300
ccagttcgcc ggcatacagc gactcgagga ccttgggacc caacttctcc aactaccaa	360
tggcctggtt gaagttgaag ctcgcgctca gatcctccag cttggccttc cgcttgccct	420
gtcctcaat caaactgatg ttgggcctat cccgggtggt cacgtgctcc gtttcgatgt	480
tgtaggccag agatccatcg gtgttcaagt agaccacgc caaacgctg ctcttggtcg	540
aggattcggc actgtcgggc gccagcaggg tctggaagat ttcgcagctg gtcgggtca	600
cgatgtgtcc ctggatgcgc agatgtgggt acttcttgga ctccacggtc	650

<210> 105  
 <211> 1630  
 <212> DNA  
 <213> Pig



<400> 105

ggtgttgcac	ctgctgtggc	tcagaccctt	gctgtggcac	aggggtccatc	cttagcccag	60
aaacttgcac	atgccacagg	tgcagccaaa	agaaaattct	tactaataag	ttgttcattt	120
gcctttacgt	agagtggcat	caaacagcaa	atttaaaaca	ccatctatca	atacatagac	180
cgcggtcaaa	gggaaagaac	tttctatttc	agcaccttta	acatggcttt	gcccgaattt	240
gggaccaggg	tgtgtgtttt	tcctctctcc	ctgcagggtg	tccccagatg	accaggccgg	300
tcctggggcgg	gaggagccgg	actgtggatc	cagttgcttc	ccaagacagg	ctgacaggag	360
agcagcaagg	gccaccccc	accgaaacca	aagccagaac	gagcagaaa	atgccgtctt	420
ccaagtgggg	gctgggagct	tcctcccatc	ctccggagcc	gtgaggctgc	cctggagctg	480
gcaggagcca	cagaggacct	ggctttgacc	gcccctctgg	gaccacaat	caggaccctg	540
actcagatgc	tgaggggcct	ggacaacacc	ccaggacctt	gctgcttccc	cagaaccgct	600
gtgtccatca	aggtccagat	ggcaccctg	tccccactgg	agcacgcact	ccgtggggca	660
ggctttccct	tgggcaccga	tgcaccttga	gggcagagac	ggggcccaat	aaacgtttcc	720
aaaccagtgg	gtgagggacc	cgaccggccc	gacacggcag	cccggatgca	gggactccgt	780
gcttggccca	gcctcccttg	gggtggtcct	gtgtcctcag	gggtggatag	gccatcatgt	840
gggtggcctc	tggggacatc	cgttctctga	ttgggtgagt	ttcagccaca	gagatattcc	900
caggactaca	aagctgggtc	ccttggggca	cctgctgtca	caaaaagaca	aggccctgac	960
cccagtagc	caagttcccc	caggggctcc	ccagggctctg	gtcatccaga	ctgtgccagc	1020
cgtgctgccc	gccccagtc	tgctgaccc	gagtctctgt	aaacatcccc	cggccccacc	1080
cagctttacc	ccaaggccga	aagcaccagc	ccccctgcac	cacagatgag	gcccccatgg	1140
ctccccgacc	taacttctgt	ctgcagttgg	ctttcagcct	cgggtggggg	caaggcctgc	1200
atctcaggct	cccgggagaa	gttgctgcct	ccacagcaga	gccagggggc	tgctgaccac	1260
ctgggcccgg	tcggatctgg	tctagaatgc	tgctaagggtg	tccttgacag	cagccccggg	1320
cggccccgcc	ctccaggaag	gaagggggaca	ttgccaggac	tcaggaatga	agccatccca	1380
ggttttgaat	ccccggtccc	accaccttcc	acctctgacc	tcaggcacct	cggctttcag	1440
agctgccctt	tctgactctg	ggacacgggg	ctgtgaggcg	ctctcgggtg	gtgacagctg	1500
ggggggggca	ctctctaacg	aggggtgggg	tgcccagggtg	actgaccaca	gccctttcct	1560

ctctcaaaaa cgcccgcccg agtgacctca cgggaggcag ggccaggaac cccaaaccaa 1620  
accagaatca 1630

<210> 106  
<211> 1820  
<212> DNA  
<213> Pig

<220>  
<221> misc\_feature  
<222> (223)..(260)  
<223> Microsatellite PIGQTL3: (CA)19

<400> 106  
agtgagccct gcaggacagt ctgctgaggg gtgtctgggc tcctcagagg ctcatggcca 60  
cgggcactgg gaggatagca ggtggacccc tgcattcagg tcccaggctc caggctccag 120  
acccccggac aggctttcta tctgcaggag gggggctcct ggggcagcag ggatgtggct 180  
gtgaggcctc gtcagtctcc ctgtttctat ctctctctgt atcacacaca cacacacaca 240  
cacacacaca cacacacaca cgcacgcacg cacacacaca gaggcgtgac cagggtgca 300  
gacagggcca tgggaggact gcccggcagt gacccagat ggccacacgg tggggccctc 360  
gtcccacttt tgctgtgat gcttccgcc aggtgtgtgg gagcaagcac tagcttccca 420  
gggctctgac cagagaggga tgggaggggt catgggtcaa caggcgccag ggaatgggga 480  
ataggatctg aggggcgggg gcaaggggcc caggcgaggc tgcagtgcc agagctccct 540  
gcacctgcag gaccagccac aggccaacag ctgcaggcag agcagggctg ctctgtccc 600  
cagaagctgg cacagcacat ggggtctgac agccccacc cgggcctccc acagaggggc 660  
gggtccccca aactcctccc ccgtcccacc tcacagctca gcatctccac tgcttgagga 720  
cgagcccaac acacgggcac acacacacat gcacgcacac acatgaatgc acctgcaagc 780  
acacactcac acgtaagcag gtacacacat gcatgcacac aatgaacaca catgcacgca 840  
cacacgatg cacacacgca cacacactca aacacgtaca tgcaagcaca tgctggctct 900  
ttgtccccgt ggaggggagg atggaggccc agcccgctggg gagggcatgt ggagtgttgg 960  
ggggctggct ccaacgccct cgctcaacag gcaccaacgc tggactgaga taagccgggg 1020  
cgctggctcc cttggggccg ctgagcaggt ttgacgcca ccacaggtgg cactgcctct 1080

ttcagaagac ggatgtggcc atgccaccct cacagcctca ccagtecccc ctcagcttta	1140
gtggtgtccc tgtcactgta cccggggcct tccttcttcc agggccaaaa gcgagttcag	1200
gggacagtgg cgtccccata attactcacc caggggtgctg tcctctgtgg tggccttgag	1260
gccaaggtgc tcccatgggg gccacaggg ctggcagggg cacttcctga gagcaccag	1320
ggccaggggg gtggcccagg cctggccggg ccccatctgg aatgagggcc ttgcgcagag	1380
gcggtgcacc cctctttaca gcagccccgg gggagagtga ctcttcgctc atggacctgg	1440
gggctgacct gtcacgtgtc tcgcccagtt gcaccccatc catttcggg tggaaggag	1500
aaagccatcc tggctgtctc agaggacctc tggagcctct tggccccagc agcccagccc	1560
ctccccgggc cgcctcctct gccacccaa aatcacctgt gccacaggg tccccctctg	1620
ggtgtccagg gcgaccaga actgcccctg cagacacacc cagcccagga catggccgcc	1680
ttgcggggcc tgtctgcctg gggcagcctg actgccacag acaggccgct tggaggacca	1740
tctgcctgag cccccaaggc acatcccacg gggcccacac agccagcgcc tgtagacgat	1800
gccacttggg gtgggggggag	1820

<210> 107  
 <211> 1500  
 <212> DNA  
 <213> Pig

<400> 107	
tgccgaatag aggtggaac caagaccga aaaaatgtcc acatttttca attattagaa	60
atttagaaaa atattttaca ggagttaaaa ggtattccat tctgggggcg ggtgggcatg	120
cccacggcat gcaggcattc cccgaccagc gactgaactc gagccacggc agtcaccatg	180
ctggatcctt aacctgctga gcccctgggc aactccagac actccatatt catgtaaact	240
atTTTTTaaC caaaaaaatg acaaagcttt tcaaaacaaa acacatttca tgggaagagt	300
ggcattgctt cacgcctgga tggctgctgc ggcttgcggg acgacgaggg cccccgcggg	360
agcgcctccg cacggcgcat caggacgtgg tgtccaggga agcgggggtca cttcacggcc	420
tctcgggtgc gcgtgggttt ctttttcggc accacacccg gactcagcac ttgggggttc	480
ttaaactgta gaggcactgc ggggctcgaa gccacatcac tgacctctc agactctgtt	540
atgtgaaaac ccatccgtcc acgagaccaa agagacagac gaacaaacgc aaggtggcgc	600

ctaggttggg	cacagcatga	gggcagagcg	gaaaccttgg	cgaaatcccg	gcgaagcctg	660
gacgtcgcca	gctcttactt	gacgcaaaca	tagggggatt	caggaactct	ctttaccgca	720
tttgcaatta	atttgctgca	aatctaaaat	cgttccaagc	acaatgctca	ctgcatggaa	780
aaaccaggg	gtaggtctcg	cccgatcagg	atgttttccc	gtgccctctg	tgcggtgct	840
gccccctg	ctggtcagt	agaagtgtcc	ctccaccgac	gacatgaaac	ttcccaggtc	900
cacgtctct	gctgtcctgg	acgaaaactc	atctctgtga	atctcccgcc	agctccgcgg	960
gagccttcca	gggctggaag	gacggccgtc	ccgttccagg	gggcagggtg	acgcttccca	1020
aagctccgcg	tcctgctagg	acgctcagac	ggcatcacc	acaaaccca	cgaactgttt	1080
ccctcgaggc	gacaggctcg	cccttctccg	agaaagcagc	ccgcacacgt	cagcaagggg	1140
ccagctgcgt	ttgtaactca	aatggccaca	tagagtttgt	cctggaggca	cggggtctgt	1200
ctgggcccga	ccactgcaca	cgcagaatat	gctgggacac	gctccggggg	ccagcttcat	1260
ggaattaata	aagtttactg	cttcaccaag	tacattctta	agtgtagctg	gccgccagcc	1320
tggggtccg	ctccgaggct	gcctctctgc	ctggaacctt	tgtgctgggg	gacctctctt	1380
ccagccccac	cccagccccg	agcccaggca	acatccttct	tgtaagacac	ccgctaccct	1440
gccctcccgc	ttctcttctt	ctggatccaa	tctcctccgc	ttctaagctc	tcttgaggct	1500

<210> 108  
 <211> 550  
 <212> DNA  
 <213> Pig

<400> 108						
atggcactcg	cggttgtgac	tgagctaccg	gacggcgcca	gcagggccac	gagggcgaca	60
agcgcggggc	tgagaacctg	tgcgagggca	ggccccctgc	gctgcagaca	agcctctatc	120
gcagggccac	agacaggagc	ccccgtgtga	ccctcaggct	gcgagaccaa	agtcacggct	180
ctgctgggaa	aacctcgaac	ctgatgactg	ggcgggtgac	cccaggacct	tgaattccgg	240
cctctgcaga	acgctctgag	cctacgggag	tgcccaccct	ctcggttagg	gcctgtgtcc	300
ttccctggct	tccagcctag	agcaaaagca	ttaaatacaca	gtgtggccca	gcccggaccg	360
tgcaggacct	tagacaaaag	aggagggaga	gagagatgag	gcagagaggc	agagagacag	420
aggtggagag	acagatagac	agagacagag	gcagagagag	agacagacag	acagagacag	480

aggcggagag acagacagag acagaggtgg agagacaggc agacagagac agaggccgag 540  
agagagacag 550

<210> 109  
<211> 900  
<212> DNA  
<213> Pig

<400> 109  
tttctaaact ctcttactag ttctagtttt ctattgtttt ctgggggggt tctatataaa 60  
cattcgtgtc gtgattggag atggttttgt tttttcctct ccaaactgta tgccatgtgt 120  
ttcttttttct tgtcttatca cactggctag gacttccagt aaaacactag atatgaacaa 180  
tgagaggaga gccaggcctt cttctcagtc ttggaggaaa cagtcagtc ttcctcattt 240  
agaatgagag cttttctttt cttttctttc tttctttctt tttttttttt ttaatagggt 300  
aaggaacttc tcttgtattc ttattttttt agagtgttta tttttttttt ctctcttttt 360  
agggctgcac ccgaggcata tggaggttct aaggctgggg tcgaattgga gctacagtcg 420  
atggcctacg ccacagcaat gtgagatctg agccacatct ggcacctata ccacagctca 480  
cagcaatgtc agatgggtta cccactgaac aaggccaggg attgagcccg catcctcatg 540  
gatgccagtc agtttcgtga ccgctgagcc atgaagggaa cttccaataa tgcaccaatt 600  
ttaaataaaa aagacaaagc atccagccca cagcctgagt aaggagttag gaggcctgac 660  
ccctgcgtgg tcctgggcct gggcctgggc tggctgggggt ggggggggggt gggggggacc 720  
ctgtggaccc tccctcctca gccaggcctg cccctccatc cctagctgtc gggggctcgg 780  
aggaaggcgg gtggatgacg gtccctggga cccctcctca tatgtatctg ggtccctggt 840  
ccctctgagg ccaggtcag gtcattgggag tcaaaggta gccaaagggg tagcccagag 900

<210> 110  
<211> 950  
<212> DNA  
<213> Pig

<400> 110  
taaccactg accgaggcca gggatcaaac ctgcaacctc atgcttccta gtcgggttcgg 60  
taaccactgc gccacaacgg gaactccttt gcttttgttt ttaggatttc acatacacgt 120

gataacgtgc cgtatatttc tttctcatct gaattatttc acttagccta agcccttcag	180
ggatccatcca tgggtgctggg agtggcagga tttgcttctt tttttttttt tttttgtggc	240
tgaaaatcag tccaggatta tcttcttttt ctgttcatct gtggaggaca caggctgcgt	300
ccgtgtgacg ctctgccggg aatacggggg ccgatcgctt tctgagccag tgttctcatt	360
ttcttgggag aagtaccggg agtggaacgg ctgggtcgtc ctgcagttct gtgctgcatt	420
ttttgaagac gctcggagcg ctttccacag tggctgcacc gactgacatt cccaccgaag	480
tgcacggatt tccccatcct ttttccacgt tttccccgca cttgctatct ttgccctgtg	540
gatgtcggcc tctccgtcag gtgtgagggg agtctccgtg cggcccaggc gaggagcgac	600
cgtgagcgtc gtttcacgtt cctgttgggc cacctgcgtg gcttctccgg aaaaagggt	660
gttcaggctt cttgcccatt tctcagtcgt attgtttggg ggggttgctg ttgagttgtg	720
tgagttccgc acgtatgggg ggcatacaacc ctttatcagc tatgagattg gcaagtccgt	780
tctcccatgt tccgccggcc gccttggcac gtgtgggcgg tctccttggc tcttcttgg	840
tgcagaaggg ttcgggtctga tgtgggcccc tttgtttatc ttcttttctt tctcaccgt	900
tgttttgatg tcagatgcaa aaatccattg ccagggtctg tgccgagAAC	950

<210> 111  
 <211> 306  
 <212> DNA  
 <213> Pig

<400> 111	
cgccacctca atcgccggtt tgttctgcaa cacgggtccag ataaccagcg cacctaacag	60
gtcgaacact gccagaactg cgaacagcgg gctgaagccg atggtgtcag ccagtgcacc	120
gacaaccagc gcaaacagcg tacttgccag ccatgcggac atcccgggta aaccgtttgc	180
cgttgccact tcgttacgac caaacacatc ggaagagagc gtaatcagcg cgccagacag	240
tgccctggtgg gcaaaaccac cgatacacag cagcataatt gcgacatacg ggttggtgaa	300
caggcc	306

<210> 112  
 <211> 800  
 <212> DNA  
 <213> Pig

<400> 112

gttttccatg atgcaccagg ggggccggga ccgcagcagg gaaggctcca tcttggctct 60  
gtaagacctt gaaaacacct cattcctctg gtcttggcct gctcttcggt acgccaagtt 120  
gctgagactg atgtggggat cagtggggag caggaatctt tctgattcag ccgtttcaaa 180  
gtgtcccaag cagaagctgt gatggcaatg ccaaggctat ccatggaggt ggctgtgcca 240  
ggggcccat ttcctgggag ccattccag gaaaggaatc ttgtagcccc aggctccagc 300  
agccagtgcg cggcccctgg gactatccgg gtagatcaga gggaggaaca gagctgtgga 360  
tggttaagcag gtggcccaag tccaatttat gtctgtggtc ccagcagggt gcccaggagg 420  
cccctcgtaa ctcttaagaa tcttggctctg gtcagctaaa ttgtatgacc attgtactga 480  
gcacacatcc cgtttaagta gaattttcaa ggatgactag gagtttgcca cctgaaggca 540  
ggaagggcat tccaggcaga gggtagagag gtgagaggga ggctctgaca ctttgggcgt 600  
gcaggggggt tgatgtgact gcagctggca cacagtgtat gcccaggcct ggcacggctg 660  
tgttgggtgt tggagaggaa gggagagggt agttgagccc aaggctctcc aggccaaaag 720  
actgaagggt accgcggctg tccggggctg gccgcagac caggaggag cagggtgggag 780  
ctggctcttg ttcgggggac 800

<210> 113

<211> 3062

<212> DNA

<213> Pig

<400> 113

cacacccag gagaggaaag acccacacag tcttgatgac agcttggctc ggggctggag 60  
ccccgagtta taaatgtcca tcacgagctg tgttctgtca gagccatcag tgggaaggcc 120  
aggccagctc agcagcccaa aaatgaagag ctaggtctgg gattgggcc aagcagaggg 180  
cacaggaaag ccacataaac aaggcaccca accccctgt catccaccaa tgtcacattc 240  
aggtcacacc cctggtcttc gggggagggt ccctaagatc cgggtggcagg gggaggaaaa 300  
gtctgactgg attccttgac aggtgtatca gcggaaggcc aggaggagtg ctcgggcact 360  
gccacctccc aggggcatga tggatcatga ccagatggca gttatgggag gaacctcccc 420  
cgtggtcaga gctctgggtg ctgtacctgg tcatgcattt cgagtggag gaaaagaaaa 480  
catacaactc cacccccagc agcttttaggc tgttgggtcta aaggctctgc ctctggaag 540

agacacgcct ctgtcagcgg acactgctaa acctaaagga agaactgcca cctggtcacg	600
ggacttccta ggccaaccaa cctacaggtg acggcccggga gcatcacgag gaggtagggg	660
acgggaaggg atgcatttgc tgctcagcgg atccactggg gcgtttcttg agccccacg	720
cccacacttt actgcaaattg cacaagcccc aggcagcagg acaagtcaca gtagctcttg	780
gttatccaag gagtcagggga cctacctgga agagtctaga acaggtgaca gaggagggag	840
aggatggtac cagcagtata gggagaatca gaaatctgac ccaccctggg ggctgactg	900
actcccagac caaatgccac actcaggttc cccgtctgcc tgcacttcca gggctgggcc	960
acgggagtta tgggccccag gtagcatcag aggtcccag gtacaggcac aagcagcaac	1020
cacaggaggg atccaggcca gggagcatcc aagaagcagc agaagctcca ccttaggtac	1080
agttctggca cctccaagtt gagaacatgt cctagacagt gcctgacccc aaccaatgg	1140
agtgtctggg actagactag gcacgccatt ttgggtccag gttgccccat ctgtacaaag	1200
ggtgtgcggc ccccaggggg acacaatgag ctcccatggg aagggtcttg cgaatctcct	1260
tagaagcaga tgtaagaggt gacgtccagc ttgtgcctgg gatgtagaag tggaaaaagc	1320
acccctcccc cgacaaggat gaaagcaaga ggcacaaaac aacctgaaat tcccaacgcc	1380
cctggagatc cttggagaac tgggattctc cacctgtagg ggcacctgtg aggagaggct	1440
gtgtgagcac ctgctgacct ggcacagagg atgcccaata ctaagaagca tcagctaaaa	1500
gtctccagga attcctggaa gctgaggaag ggctcaggag agggtagaga agccctgggg	1560
ctatagatat aagggacgtg cacacccact tgcagggtccc catggacccc agggacattc	1620
acagtgatgg gcaagattcc caaaatgcac cccttgtgtg tgggcctggg tcggtgggtc	1680
agcagacacc acaccaaag cacaagcac acaccctcag gctactctcc tccctctccc	1740
ttgtggaaca tgagccttga gatgctgggg cacgtgaaaa acactgtcac acttaggtcc	1800
tggtgaaaac tgactgcggc cagcgggaaag aatcataaag accctacacc cacacacagc	1860
cttaattaca gctgtgagtg gggctggagc cccaagaatg tctacaccca taagacatag	1920
cgттаатсag ааааасаага асagccccaa cccaccacc aggctgacaa cтаacaggтс	1980
atgttggaat atcactggga atgttctagg agtgtagaaa gacacaccaa ctagggcatg	2040
atgcaaagat aatacttcag cctgggagtg gatgtgacac agggaaaagc ataaagtgat	2100



ggcagaggac	tttgatgtca	gtgatggaag	ccacaaaaac	ttctagctta	gctccattcc	2160
caacaagatt	gactgcaaac	cccatgctaa	aacaacagca	aaaagaaaga	atcctcattt	2220
ccaggcataa	aatTTTTTccc	ccagtctctg	ctgtcctcca	taagatgtct	gatttcaaca	2280
ggaattacga	ggctataaga	aaggcaagaa	aaaactacac	actgtcaaga	gaaagccatc	2340
agaataacca	gactcgtagc	acagacactg	gaattgtcag	gatattttta	ataaccgtga	2400
caaatacatt	aaagatttcta	atgagaaggg	ggtagacatg	taagatcaca	tagatttcag	2460
caaagagatg	aaactcgaag	gaaaattaaa	tgggagccct	agagtgaaaa	acactgtagc	2520
agagaagatg	ggttcatccg	taaacatgac	acagcttagg	aaagaatcag	tgaacttgaa	2580
gacagggcca	cagaaaatat	ccaaactgaa	atgcaaggag	gaaaaataat	gaaaggggga	2640
gagagaaaaa	ataaaagaac	aaagcatcca	agagctggag	ggtgacactg	aagaagagag	2700
cataggcata	gctggaatct	cagaaagaga	gaaagaaata	acccaagatg	taatggatga	2760
gaatttcaca	gaagcgttgt	caagcaacaa	accatacatc	caagaagctc	agagaacacc	2820
aagcaaggta	agtactgtaa	aaaaatagcc	cgaggataac	ctcattcagg	ctgctgaaaa	2880
tccatgacaa	aagaagtctt	gaaagtagcc	agaaacagaa	ggcgtgttcc	attcagaggg	2940
aaaagacacc	attgttgcca	gaaaccaa	aaaccagggc	tgaaggggta	aaactttttt	3000
tttttttttt	ttttttttgg	ccatgcctgt	ggcatgtgga	ggtttcccga	tcagggatca	3060
ac						3062

<210> 114  
 <211> 1300  
 <212> DNA  
 <213> Pig

<400> 114						
aaacggataa	atacaggtga	cccacaggca	gaagctgaag	tacaaacagt	tcacaacggc	60
acccaaaaaa	taccgaaggc	tcaagggtaa	atctgacccc	agatgaaagg	ccttctcacg	120
gaaaatggca	aagtggcgct	gagaggcatg	agaggttcga	atagatggag	ggctccgccg	180
ttttcccggg	tccgaggatt	cagtgaagtc	acgacgcaa	ttcctctgaa	acgcctctct	240
aggttcagtg	cagcccagac	ccactggcag	ccgccctcgc	tgacagagaca	gcccagctgg	300
gtcttgaggt	tcctacagcg	aagcaaaggg	tctagaaaaa	gcagacgtct	ctggaaaggg	360

agaagcagcc gatggattgg catacggcga caggagattc ctoggacagt ggcaccagga	420
gaggggtgga cagagactgg tgcaaccgag cgggcccagg aataagtcca caccacacg	480
taccatctcg ttgtttatth atthtttctt tttcagggcc actcctgggg catgtggagg	540
ctccccagcc aggagtcgaa tcggagctgc agctacaagc ctaccccaca gccacagcga	600
cacaggatct gagccatgtc tgcagcctac accacagctc cgggcaatat tggatcctta	660
accactgag caaggccagg gactgaacct acgtgctcat ggatactagt tgggtttgtt	720
accactgagt cacagtggga actcctthaa tthtaattth tgaaggthca gaactctthta	780
atthttthagt gaggtataga ttatattacg caccattthct ttctgacttc ggtgcacggc	840
thttcaacaa atgggtgctg gacctgctgg gtgcctthct caaatgaacc acaagccctc	900
cctcgcgccg tatgcaaaat ttaactcgag gggctcatag acataaacgt aaactctaaa	960
gctataaaat thccagaaga aaacgtaagg aaaacctthg gggctctggg caaagattth	1020
thacccatga cagcaaaatt acaatctaca gaagaactgg tggcctthtat cggcattthaa	1080
aacacctgcc cthtgaatga tgctgtcgca aaaccgaaca tgcagcaaaa cggatgcaac	1140
tagcaggtct cacactcagt gacctcagtc agaaaggga agacacgcca cgtgacatcc	1200
cttagatgca gaatgtaaaa cacggccccc gtgaaccgac ctcaagagag agacagacct	1260
acagacgcag caaattthggg gttgccgagg gggatgccgg	1300

<210> 115  
 <211> 3000  
 <212> DNA  
 <213> Pig

<400> 115	
tgtgagaccc cttggcgggc caggaccccc caaggcgacc gaaggcctca gcgccccag	60
ccgccccatc cccctctthc ccgacacagg atthttthtcc caccaagctc tgttcccttg	120
gtcacgtctc cacttgagca gcctcagggt ctcccgggtc ctgtatccac gacagcgtga	180
ccttcttggt gtgtcaacct aggaccccac gctggccagc cacgcctthc cagagcaccc	240
ccgccccatc tcagagtcca gaggaaggc cccattgac ccagaaaacc aaaacgcaga	300
gactctggga cgccagcaag aacgtacact gactcccacc tgcttcaggc acggaggcag	360
gggtgggtta tgagcgacct cgtggaaggc ccttcttgct catcgagggg cttccagggg	420

ctcctagacg gggatgagtg tggcaacatg tgcgcgcatt acaaaagacc ctgcagtgct	480
gctgggatgg gtcccccggc tagaaaagca aaggattcca gccagtcga gtaggagggc	540
gcctcggagg ctgcagagggc gcggggggcg ctgaccacca ctcggaagc cccgtgttgg	600
aggggacgcc cggccccggt gcagccggtg cgcctccgga taagctcta agaggccgcg	660
tgccccatgc acgcgcgtgc acacactcgc tgcccgaggg tccttcagca cagaccttgt	720
ggggacggag gacctggcag ggggtgtggct ctggggaagg ggtctgtccc aggaacctg	780
ttctggattt ggggggtgggc gtggatatcc cgtcccaacc tacagaaggg aggggcttaa	840
aaagagcccc tttggtgtga ggggccagca atcctttggc tttttcttgg ccacttgga	900
gcttgacgtc tggtcagtga ctgggagcca gggccagagg ggggcagccg ggctgaggca	960
ggttcaggcc aaccatctct cggccacact cccgaggtcg ggcagctacg gggccccag	1020
agacacaagc ccaggggtc cttccccccc gccccctgcc ccagatcacc aggagacca	1080
agcagctctg cctccccgtg cctgagaaat gcccctctg ggtaccctaa tcacctccc	1140
agaaggtaga gtggggggcc caggacaggg ggaccccagt tacagagccc caggcaggct	1200
tcccaggggc gaggggactc cgtttggggc acagacggag gcagagcggg ctgatggatt	1260
ctcccccggt tcagggatgc tggctgcctg gcctccagga gccggcggtg ccatctgatc	1320
tgattaaggc ctgcagtccc agctgggcgg gcacagcctg ggggctcggc gggcaggga	1380
gaaggcgctg tcgccccagc cggtcaggct cgctttctct tcatttcctc tccattaaaa	1440
gtgtcagaac catttattga ttttttaa atcaggacgtgc tgtccgtgac acagcaaagt	1500
gaacaaaatc agagcaaaga gaggccaggg ctgaagcccc agagggcggc gcctccaatc	1560
cgggttgtgc cccggggctc caagcccctt cttcttctgg ggtcctgggc gtagtgcca	1620
gggcagaatg cacctgccgt catcctggga ggcttgcca tcgctggctt ctgtctcatg	1680
acgcaccgtc gttccatatc tacggaaaca gcttcgcatt aacaggcagg ggaggcgggt	1740
gtttctcctt tatctgcca ccatcggcgc tggggccacg tggagcccag ccggtgact	1800
tcccgctcgc acgcaggga ctgattgcag gaacgaggac atccagcccc cgctctcaa	1860
tgccccgggt gctgagagca ttctgcccac acggcttggg tgggacaagg gatggagctg	1920
tgcgccaggg gcctggctgg ggcagaaggg ggctgcccg tgtctgcccg tggcctccag	1980
cacctcggc tgccaggctg ctctggagag gtgccccggg gccgagggcc aggggcaccc	2040

tggtctgccc	cacgtctctc	tgctctgctg	aaagttccac	cagacgcgtg	ctataccctg	2100
ggagtcagga	ggatggggga	tagttggggc	ttgacgtctg	tttctgaaaa	aacaccgttt	2160
tccctgaaat	atatatgtat	taatttttctg	tcaagataaa	actgtgtata	gtttttcgtg	2220
atgagaaaac	gcatccatct	tccttagaaa	gcctgaagag	gtacaggagc	ctataaagga	2280
caagatgaca	gatgcctcta	acgcacacca	aatgtgcggt	ggcccccagg	ggaccgcata	2340
gacggggcgg	ctccagatgg	ccaccgtgtg	cgagggacac	ggttcagggt	ggcagagtat	2400
tcctgggggg	ggggggctca	gcggttccca	tttccccctc	ccttccttcc	ttcatttctt	2460
tccttctttc	tttctttttg	tggttttagg	gccgcacccg	cggcgtgtgg	aggttcccag	2520
cctaggggtc	taatcagagc	tacagctgcc	ggcctccacc	acagctcacg	gcaacgccgg	2580
atccttaacc	cacggagcga	gaccagggat	ggaacctggg	acctcatgga	tcttagttgg	2640
gtttgttccc	gctgagccac	aacgggaact	ccagccattc	ccatttcttg	ctccagttcc	2700
aagaattcca	attcttattc	ctgttcttta	aggccagagg	cgacagccac	gccgagtccc	2760
agaagcaggg	ctcaaggatg	ctgctgttga	ctgtgtccgt	gggggggggg	agttgataag	2820
aacccccaac	acaggggtgg	ggccagcaac	gggggagggg	ggaggggggg	tggtggggaa	2880
aagtcacctg	aaccccatgg	gctgccccct	ccaggctggg	gcacgacccc	gagcccatg	2940
gcccgaggag	aaacggtccc	agccccaggc	tgggctcccc	cacctctgcc	ctgacccgc	3000

<210> 116  
 <211> 1895  
 <212> DNA  
 <213> Pig

<400>	116	
tcattggaagc	ccttatcaca	acctcggatc
caaaacccac	tgcgcgagtc	cagggataga
		60
actcgcattc	ccacagacc	tatgttgggg
tcttaaccag	ctgagccaca	tggaaactgg
		120
gtaatctatt	tttagatgtt	cctaggggtt
ttggccttgc	ctgtacgtgg	ggacgtgct
		180
gggccaggga	tcaaaccgc	gccacagctg
tgaccaagc	agagcagtga	cagcaccgga
		240
tccttaagca	cgaggccagc	aggagcccc
tgtgtttaga	ttttggtgag	gatactgcgt
		300
gggattcagg	atattcactt	tggggctggt
ggaattgccc	gtcgctgttt	aagcaaagag
		360
aaatcccttc	actctgtgta	actgtgggga
aatcctttag	tctcttgaaa	ccattgcgtg
		420

tgtttaagag	tggtactct	gccaccataa	atgccagac	cagcgcttc	ctgagatccg	480
cttttgttgc	aaatatctgg	tttgaatgct	ttgatcgccc	gcaccagacc	aggggtggg	540
gacgccgccc	gggacccgac	gtgaccatcg	tgcttctgta	tccgcccttt	ctccggcacg	600
cgccccctgg	ttgectctgg	ctgcttttag	tggaggaact	gaagcctcgc	caccagacc	660
ccgagaccgc	aggaccaca	atgcttcaaa	cacctgccct	ctgactttta	caggtcaagt	720
tcgccaacgc	cgaatttgca	ccgattggct	acagagagca	cgggtggcgcc	aagcctccac	780
ttggagtttt	ataaggtctc	cctccagctc	gcaatgaaaa	tgagctgtga	taaggcaaag	840
acaaaattag	tatgaaatcc	agatgcttca	tctacaatac	aatgaccgcg	ggatttgggt	900
ctgagcgact	gaaatcaagg	tgggcttccg	gagggaggct	gttagaggaa	aggcattcac	960
ggaggctcag	gtccgagagg	cttccacacc	cctaagaggg	ctgagacggc	aagtagggac	1020
caagccccgc	agtcgggaga	gctgggcagg	aagggaagtct	gaggtcaccc	ccacctgggy	1080
aggaactgcc	tagagaagcg	ggggcgggaa	gcaggggatg	cccagtcca	agacagggac	1140
agggcgga	gggctctctg	caggccctca	atgctgccac	agtgtcctcg	taagagggag	1200
gcagagagaa	ttgacaccgg	ggagaccacg	ggaccacgga	ggtggagacc	gggctgccc	1260
cgcggtgccag	ttgctccga	agccggcccc	tccccagag	cctttgggaa	gaggcgccaa	1320
cctgcagttc	tgctactcgg	ggacagggac	agggacagcc	ccctggagcc	gcctcttagg	1380
ggcagcatcc	cccagaacct	tccttaacag	accatctgga	gagagatggg	tctgggctgc	1440
agctcctgga	actgttttgc	ccaccggcg	agcaccagtg	ggtgccagcc	tgggctgccc	1500
agcctcaggg	ccggggaggg	ctgagggcac	tggggcccgg	ctctgggact	ccctgcctc	1560
ctgcccgtgc	aggacagcca	cctcccagca	tctgcttcct	gccaccaca	tcccaggac	1620
cgtcagccca	ggcatgcccc	tggcgtcggc	cactcacacc	acaggccagg	aaccaagg	1680
ggcaacacag	aagggcagtt	gccatctgca	gatggaatgg	acaaactggg	gtccgtgatg	1740
atggcaggct	ctgggcgccc	gggctggcag	gggagccagg	actgtgcggc	catcacagga	1800
agggcatgac	ggggtgaaag	caagagtgga	aacctctgcc	accgcctgg	gcgcacatac	1860
cggccacct	gcagcccac	ccccatttgt	ttgct			1895

<210> 117

<211> 1040

<212> DNA

<213> Pig

<400> 117

gcgcgcgcgga tccttaatta agtctgagag atctgcggcc gcggccaggg tctgcttctg 60  
gccaagtgtg gggctctgct ccacctggc tcggaggtcc acccatggca aagcctgggg 120  
tcctcccact gaatatttgg gggccactc gtgccaaagg ctgggtgtcc agtgtgcaa 180  
cggtagatgg aagcaatgtc ttccaagga ccgtccaagg tgtggtcagg cctggacagc 240  
tgtgagtccc ttccgggacta gacttggtgg ccgaacccta gggaccgtgc ccgagggccc 300  
ccacgaggcc aggtgtttgc ccagggaca gaacggccaa ggggtggccga gggttctttt 360  
tgtttgtttt ttcttctttc tctttttctt tggccgaggg ttcttaaagc gctctctctg 420  
ctctttgtcc cgatcctgag cgggcagtgt cctggtcggt ggggtgctgg gcagccgcag 480  
cagggctgag agagcccggc ttgtcactag ggcgcgccgg tgagcccagc gggcatgccg 540  
tgtccagacg ttggatgggg cagcgagggg actgggggtgc ccagccccc gtgggaagcc 600  
cgccctgtgg aagccgctgt gtcgcgcaca acaagcaccg tcgactagct ggtgaatcag 660  
cgcccgctgc ccgcgtaatc ccaggcgctt tctgcccac ctgagccctg accccacacc 720  
ccttgcgacc gctccgtgga ccctggggcg atgaggtgaa ccgtgggctt ggccatcgtg 780  
gtggcagacg gtggcacacc cgtgcgcctg tcggcccccc tccatccagg agcagagtgc 840  
gcaccacagt ggggctgggc agggagccgc ctccacctcc gccctgaggg gacgggactc 900  
tttcgacccg gagtgggaag ggacatatgc ggacgatgcc agaccctgtc tgtgggggga 960  
gggggagaag gccctctttg gagaattcca ggacgggtga ggaacgtgtg ctggaccggc 1020  
cgggtcggag gtgggccttg 1040

<210> 118

<211> 9236

<212> DNA

<213> Pig

<400> 118

ggcaaccagg ggaagatggg gaagcggggg gcaggggctg ttgcgcgggc caaggaccac 60  
cttggaatc tggagcctgg caggagcggc gcaggggtga ggggctggct tgggcagggc 120  
tggtggcac ctgggagcct ggccggggtg aggtccgggc tccaggtgc cctataggca 180

gggcaacatc	ggcatggggg	gtgacaggcc	cgagctgggg	tgcggaggga	agagggggga	240
gccaggcatt	catcccggtc	aatttttggtt	tcaggtcgtg	gctggctggtg	gtcagggggga	300
gttgagaga	ggttcgcccc	ggggcctggg	gcagcggagg	tgtagctggc	agctgtgggc	360
aggtgaggac	agccgtctgc	cgggccaggt	gagtcccctt	ccctccccag	gccttgtttc	420
tctggcctcc	tgcattccga	ggttctgggg	agcgaggggc	ggcgaggcga	agcggctgac	480
ccccggcag	agtggcggcg	gacgacaggc	aaggcgggca	gaacagggtga	cacgtctcag	540
ggggagctgg	gaccggggcg	ggctgggggg	ccggggccgt	cccagggtga	aagagcatct	600
caagcgagtc	tgggtgggaga	cgaggcaggg	ctgccagcag	ggaggagacg	caacaggcgg	660
ggggcattcc	aggcccgggt	cggacaggac	ccgtcggggg	tgtcaggaca	gtgggggtccc	720
cagccgccac	ttcaccact	gcaattcatt	tagtagcagg	tacaggagcg	gctctggccg	780
ggcctcttga	ggcctgagct	ggagcctcga	gggccggaga	atgggaaaga	aggtgcagtg	840
tgccagacag	acgtcacctg	gagggagcac	ggccgtgggg	acgggccccca	gagagatttc	900
ggcagcaggg	aggctgcgcg	ggcccagcct	gcggacgtgc	gttcccacgc	agcactgcgg	960
cccaggggct	ggcgcggcag	ggccccgggt	gtccttggtg	gcactgtgcg	ccctcgccgc	1020
tcgcccctgg	gactggcacg	gcagacagga	cagcaccacg	gggagtcaag	ggcactgacg	1080
agaccagact	aggcgaggcg	ggtgggggtg	aatggatgtg	acctctgggg	ggagggaggt	1140
ggggacgcag	gcagggggcg	ggcgccggag	cctggcgggcg	agcgaggcca	aggcgggcct	1200
ctgcgggtga	caactgagca	catatgggta	cctttgcgct	cgcaccggag	acaggtgagt	1260
gtctggcccc	ggcctgccgc	cctcccggcc	ccgccactgc	ctctgccttc	cccctcgacc	1320
aggggcctct	gcttccccac	agcctcgtct	ccagtggggg	tggacacact	gccagcacca	1380
caggccggac	gccaggatgt	gcttgagggg	acatgacaca	gtccggtgtg	acggagaggg	1440
acagacgtga	cgccgtccgg	ccttcctggt	gagcgcaggt	ccaggccttg	gccccagggc	1500
cagccgcccc	acccccacc	cctcatggcc	gttttctgtc	ccgcagaaca	ctctcggttg	1560
gccccgcggg	ggagctgcca	caccagcgt	ctgttccttt	gccttcctga	aggagcacgt	1620
gcatgactgc	tgtctcttgg	acccagaac	cctcaaacga	caaggtgagg	caggtcccgc	1680
ctcgccccac	acgtggaagg	ggcgtggggc	agagccgggc	gctcacggtg	ccccctccc	1740

cctgcagaga	tggtgctacc	cagctcatgc	ctgggccttg	gacccggact	tcttcaagtc	1800
ctcctagctc	tgactcaaga	atatgctgca	ttctggagcc	actacactac	ttgactcagg	1860
aatcagctct	ggaaggtggg	cgcgcgctcc	tcccgcctcc	ggagccccgc	ccgctgcccc	1920
ctccccgctc	acgtcctgtc	tctgtcctcg	tccgcagggt	gagccaaagg	aacagacgtc	1980
ccacaccacc	ggaccaacgg	caccgcgggg	gttccccacc	ccccgcccgg	ccactccacc	2040
tcggcggcca	ccccctgctg	cgccttgag	acaccaccag	cctccctctc	tccccctcct	2100
cctttttttc	ctctgtcttt	tctcttctct	tctttcctct	cctttgctca	gaagactcgg	2160
ggcatccagg	actctgtgtc	ccgtccttc	ctgaattaat	ttgcactaag	tcgtttgcac	2220
tggtttggag	tcctggaacc	agccccgggt	ctcggagcgg	gtgtgtgagc	tgccgagtgg	2280
cctggcctcc	tcggccccgg	ccccctcagc	acctgccatt	gtccatctct	gtctgggggt	2340
gactgggtgg	gggcctgagt	gtgtggggcc	ccgccctccc	ctctcctagt	ctggaagctc	2400
cgaccaccga	gcagacctca	aacgctgcac	tgagtgtcca	tctcgtcatg	tgccccctct	2460
cgcacggggc	acccagagc	cctggactca	tcaataaact	cagttaccgg	aatctgtctc	2520
aggggccttg	caattgggct	gggggtgcgc	cggggaaggg	ggggatgaga	tggggaacat	2580
gcaaggaagg	gcctgtgggc	tgggggacac	agaatgggtg	gggagggggc	tcacaggact	2640
cggggggtaa	tgaacgtggg	gctgggcgca	aaggggagtg	ggacgtgggg	atcagggcgg	2700
ggggcctgga	ggatgcaggg	tccctgcagg	gaaagggggc	cgagggcgtg	aggcatgtcc	2760
tcagccctga	gaggccctac	cccacaaagc	acagcctgcg	cgcgacctcc	aggcccccaa	2820
acccccgccc	cagaccctga	agccctggtc	cagggcagtg	ggtctgactg	gcggaaggaa	2880
catgccaccc	aggctggcca	caccactggg	acgcccatgg	gcggccactt	tcatcaagag	2940
cctggcaggg	cctgagtgtc	gggctggagg	gcacagaggg	tccccctccc	ctcacgcttt	3000
gcggtgctgg	ggcaccgcag	gagtgcccaa	caggagaccc	caggaagtct	gctgggctgc	3060
agcgaagggc	agggtagggg	ggcggcccac	aggggcccag	ctcagtaggc	aggtggcagt	3120
gggaggcggc	agaaagttag	aaagggtgga	ctgggcacgt	caggatctcg	tggcggcagc	3180
cccggagcca	cggccttggg	tgcactgcag	ccccacggt	tggtgtcccg	gtcccaggca	3240
gcagctgggc	tggtgacgcc	cctctgcctc	tgccacccc	cccaccgcc	cccccgccag	3300
cctcccagcc	cctgggcgcc	tggcgtgacg	ctgggaacgc	gaggagcag	gcctcgga	3360



cagggctggg	tccttgacct	cttcctctgc	tcagggcagt	caggaaatgc	ctagcggggc	3420
gactgaccga	gaggagatag	cggaggcctg	ggagaccccg	cgctcgtgcc	gttcccagcg	3480
tccggccgcg	tggcccttgg	ctggcctggt	ttgggccccca	tgagctcacc	ccccgcccc	3540
cacagcctcc	ccggtcttgg	tctcctctct	gggccctgct	gtccctcctg	acgggggaca	3600
gagccctcca	gggccccggg	gggacggtcc	cgggtcagca	gggccccgtg	gcagcacagc	3660
tgcgttttgt	gaagccccctg	cccaaagcac	cctcagcggt	tcctctgcgc	gtccggccgc	3720
ccccggaggc	tttcccaagt	ccacgggcaa	ctcgcaggcg	agccactcc	acctccatca	3780
cgcggtttg	gccagcggca	gaagcactcg	cccttcaggc	gtcaggagtt	aagccccctc	3840
aaggccccgt	gctaatacgc	tgcctctcct	ggagcttcgc	aaagccccgt	ctcagagccc	3900
agcttccccg	gggctcaccg	tgggtggcatg	ggcaccacag	gtggccggag	gggcaccgag	3960
cacgacgggg	ctgtgggggg	tggaggaggg	aggtttgtga	ctccgaacct	ctactgaggc	4020
acacagagga	cacggccgct	tccaggggag	tcagcctgcg	aagggcagag	gggctgtagc	4080
ctccccgtca	cggcctcgcc	tctgccctgg	attcctcctg	ggggcccccg	gctcgtcggg	4140
gaggtgagtg	cccctggatg	ggcgtaggct	ggggggggcag	ggagtggggg	agccccgagg	4200
ccctggggcc	acagccctgt	cttgccccac	acacagggct	gtctacactg	ggtgcccact	4260
tgtctgtctt	ctaggctgtt	ccctgggcag	ctgcctggag	ggcctgtggc	acagtgcggg	4320
cagccagtgg	ggaggccggg	gatggggccg	gggataggga	cccctgcccc	tgggtgagcc	4380
ccacctgggc	tgggaagaca	gcagcagcgc	cccttcagggt	ccatggacca	ggggaccag	4440
ggtggactgt	gtttaccttc	agcccaggcc	agtttctctg	ttgagaaagc	ccgggagggg	4500
gtgcgggaca	ggccccggcc	ccccacgcaa	aggcagtttc	gcaatgtccc	tgcgtgact	4560
gaaatgtcac	caggcacacg	gcttgaattt	ctccccaga	cctggcaggg	gcgggggtgg	4620
gggcaccggg	ctgctgggat	cttgccccct	gaacctcccc	cggccctgcg	gccagggagg	4680
gtttaggctg	agtgcagacc	cacggaaacc	tggacccgac	atgtctgtgt	gtccatgtgt	4740
gtctgtgtgt	gcgtccacct	atgcgtctgc	gtgtgtgtcc	atgtgtgtcc	acatatctgt	4800
gtccacgtgt	ctgtgtccac	gtgtctgtgt	ccacgtgtgt	gtccacgtgt	gtccatgtgt	4860
ctatgagtcc	ttgtgtgcat	ctgtgtgccc	gtgtgtctgt	gtgtctgtcc	cctgcagtcc	4920

ccgtggacct	gtctcttata	cacatctcaa	cctggcagcg	ccccttcagg	tccatggacc	4980
aggggaccca	gggtggactg	tgtttacctt	cagcccaggg	cagtttcctg	cttgagaaag	5040
cccgggaggg	ggtgcgggac	aggcccgggc	ccccacgca	aaggcagttt	cgcaatgtcc	5100
ctgcgctgac	tgaaatgtca	ccaggcacac	ggcttgaatt	tctccccag	acctggcagg	5160
ggcgggggtg	ggggcaccgg	gctgctggga	tcttggtccc	tgaacctccc	ccggccctgc	5220
ggccagggag	ggttttaggt	gagtgcagc	ccacggaaac	ctggaccgga	catgtctgtg	5280
tgtccatgtg	tgtctgtgtg	tgctccacc	tatgctctg	cgtgtgtgtc	catgtgtgtc	5340
cacatatctg	tgtccacgtg	tctgtgtcca	cgtgtctgtg	tccacgtgtg	tgtccacgtg	5400
tgtccatgtg	tctatgagtc	cttgtgtgca	tctgtgtgcc	cgtgtgtctg	tgtgtctgtc	5460
ccctgcagtc	cccgtggacc	tgtgtggtct	ctggtgtgca	gccctagccg	cggcccgtcc	5520
caggctgagt	gtccccaggg	tgacgcacag	ctgtgacgag	ggtgtgggtc	ccgttgcccg	5580
tgctgctggg	ctgtgggccc	tatcctcttt	gtggctgctc	tgcaaggcct	gatggctttt	5640
gtgtggcctg	gccgttcggg	tccatgcccc	ctggaagagc	aacgtctgag	ctagctccac	5700
gcgtgggtcc	atctcggccc	aggtttaatg	agccactttc	aggcagggat	tgcacaggag	5760
gcaggggtgg	aagtggctct	gctcagaccc	ctgaacaggg	tctggagatt	ctccaagggc	5820
acaaaagaac	ggacgatgcc	cctgggggtca	gcgacaatgc	tccctgagaa	atcttggcac	5880
acagggctgg	gcctgcgagg	tggcccctcg	ccccaccca	gcctcctgga	ggacaaccgt	5940
cgccctgctc	ccagagctgg	ggggcgccac	acgtggggca	caggagcat	gggcccgatt	6000
ccaggcctgg	gctccctctc	gtgtccagga	tctccccgtg	tcttgtctca	acaagcccct	6060
gacttgagg	cccaggggtg	accttataaa	gggggaacag	aaggttctag	aaggagcgtg	6120
gccagctttg	gcttccctag	ggctgtgggt	accacactgg	gccacggccc	aggccacccc	6180
accgcctcc	ttccccctgg	ccccctccct	tccccgcacc	tctccctggc	ctgcacctgg	6240
tgacacggct	ggctcccagc	cagggtgag	ggggaccagc	ggggcccctt	cctggaagcc	6300
cacctgcagg	ccggcttgct	gggaaggggc	ctgctcctcg	ccggccccac	ccgcccgggg	6360
ccgtttcctg	gaagcgggtca	ctggatatctt	tgttccttgt	cagcgccgag	cttgcataaa	6420
gcagacactg	agctccttgt	cctccgggag	cacgcgtccc	atcacggaac	acctggccgg	6480
acacaggcgg	gcagccgggc	ctgggggagc	agcgcgggcc	tggggccgga	ccagcaaacg	6540

atcacggcgc	cgagcgcagg	gccccgcgcc	cttctgcagg	ccgccccac	gtgcccaggc	6600
ccagcgggtgc	ccatcctgca	ggctgggagg	aggctgtggg	cgcagagctg	agaagggggc	6660
agaggcactg	gggggggaca	gccgtgttcc	cacactttgc	agaaaccttg	gccggcctgg	6720
atgtcttgct	gggagagctg	ggggagggga	cagggcagga	agccgggtccc	cccagcggg	6780
gtaggaagag	gcctcggccc	tgggaggagg	aggaggggag	ggcagtgaga	tggaaagagc	6840
accaggggct	cgaggcttct	ttctggaaca	aggactagaa	ggaggaggcc	gggcagctgc	6900
ttgggatgct	tggaacaggc	cggccccagt	gctgacaggg	acgtgacctg	ggggccggtc	6960
ccgggcccag	gcgggctggg	agggcgccctg	gtgggtcagc	gccactcaga	gccctggcag	7020
cagggggcct	gggcacggct	gcaggacaga	gctcaggaca	cagatggggg	cgaggactga	7080
gtggggcacc	acagatgctc	ccaggagggtg	gccaaaggagt	ggccttggga	tcccaggatg	7140
gccctggtcc	cagaagatgc	ggcagcccaa	gggaccaggc	cagggccgca	gggggccaca	7200
atctgagcag	ggctcaggcc	cagggcagag	gccccctccc	accagccct	ccctggggccc	7260
gcctctccgt	gcaggcagtg	ggctcagatg	gggcagacat	gagaccaggt	ccagggagaa	7320
gcggggcccc	ttggcttcat	tcaggtggct	ttcagaccgc	gccccgtgcg	tggcaaggcc	7380
cacagcgctc	aggagcacac	agacccccac	cacgggctcc	cccaggttgg	gcggtgacat	7440
cagccctgtg	tcaacagcag	gagctggcag	ctccccaccg	gggcttaggg	agcggggacc	7500
ctgagccacc	ctgccaccgc	cccacccccac	cgtggccccac	acgagggccc	gctgctctgg	7560
gtctggggcc	aaggcccccc	aggcgccctgg	cactgtctgc	ccctcccgt	ggctctccgt	7620
ctccagtgtc	cccgccagag	agcatggggc	cacaggcctg	aatgccaccc	tcttcctccc	7680
tctggagggg	gcctgaggtt	ttgggggttc	acagagtggc	ctccgggggtg	gtccaggcc	7740
cagcgaggca	aagcggaccc	cagggagtcc	cgcggaatgt	gggacagccc	ccccgtagat	7800
ctcggggggg	ccaagctctg	gttgacctcc	atcctggggc	tgtgggcctt	tggtcagtgg	7860
ggaggggtcat	gacaccagc	ccaccagctg	gtgacagccc	tggacgtgcc	ggctcagggc	7920
tggcctgccc	ctgcagcctt	gaacccctgt	tctctgggag	tgggggcgca	gggggcgccg	7980
gggcaggggtg	agagacgaga	gcctctcttc	ccagaacttc	tgcttgcgat	gaggaccag	8040
caggggcctc	tcctcaccag	agggcctctg	ccggctgcag	ggccccagag	aggcccagag	8100

gctggaggcc	gggccttggg	aagaggccg	acttccagaa	accagctgcc	cgctccgcag	8160
caccagcgc	ccacttggga	ggggggcgcg	ccccctgcc	ccgcccgggt	ccactgctgg	8220
ggccgccaca	ataaagtttg	tccctgctgg	ttactgtccg	tgtctgagag	gtttctggag	8280
cctggccaca	atggggcgtca	ggatgcggct	gggagggagc	ctcgcgagtc	agagtgtgct	8340
ggtctcggac	aggccccggc	gccccagcc	cgtgctctgt	ggacagatgg	gtgggtgggt	8400
gggtgtcggg	gggggttggg	gagggtgggc	gggacgaggg	gcttcttgca	ctctgtccca	8460
gggaagcggg	gaccaaggag	gggacagccc	ccggtcacca	ggagggtcct	gtccctctca	8520
ccccccggga	caggtgagct	ccccggagcc	gcccttctgg	gacaggaccc	cacggccagg	8580
ccacggcccc	ccccaccccg	tggtcctcc	gtcccacggc	cggcctgggg	ggccacgggc	8640
ccagggcccc	cgctccccgt	tggccctccg	aggggtgaacg	acctcgccctg	ggacgtgggg	8700
cagagggcag	gcgccaagag	tgacccctcg	ggacacgtgg	ctgtttgcag	ttctggaggc	8760
agccgagata	aagcggctgt	tttcccagtg	ggctcagggc	cagagggggg	cgaggggcag	8820
ccccagtcaa	ggccggggcg	ctgcctcggg	ctcccccttg	tgccgagggg	gggggccggg	8880
tgcacagcag	cccctgcccc	ccgcccgcgc	gccgggcgag	gcaccgtggg	acccggcctg	8940
gtgccccctc	cccgccccctg	ctcagggggc	agccctctct	ggttcccagg	acgccccgcg	9000
cccgaggcg	gccagagagt	cccagagtgt	tagcctccca	cgtgtgggat	cctgtcatat	9060
gcgacagctt	aactcaggcc	gaatttcatg	ggtcctggat	ttgggtgggc	acggccccctg	9120
cacagcgggg	ctggaagcct	aaggcgggtg	gcgtgggggt	gagaggcccg	cagacaacag	9180
gagggaggct	gggacacttc	aagggttgac	atgctatgcc	tgtcacggat	aaatgc	9236

<210> 119  
 <211> 5349  
 <212> DNA  
 <213> Pig

<400> 119	
agatgtgtat	aagagacagg ggctgggtgg gaaggacaga gggtagggcc ggaggaaatg 60
ggatgcagag	cccaccgtgc acgtctgtct ggcctttgag cctcgctgag tcgcaagaag 120
ccctcgggcc	tggaaacaga cccccggccc ccacccccac cccggcccc ggattacccc 180
ggcatggctg	gagggcccga gaagccaccc aggcttcccg tgccgagctg ggtgctgggc 240

ccagccgagc	gggcttgacg	ccacgcttag	ccctccccag	ggagcccagg	gtcgggaagga	300
agaggccggc	cggaggggccc	tggccgctca	ggctggaggg	ggcccccg	tcaggatggg	360
ccccagacgt	ccccgctccc	cggccatccg	tcacggagct	gtcaccagg	aacgtgctcc	420
agacgtgctt	tcttgccgcc	gaggccccga	gcaggctcca	ggcgccccca	ccccgaacg	480
cccacgcaca	ccctcgggtct	gcgaacaccc	tgcggtcacc	cgggtggcccc	ggttccccgc	540
gcccgcgcca	tccgggtgcc	ccttcctccc	tgggtcgggg	gccatgccct	cagcgggcac	600
gcaggcctgt	gcaggctctgt	tctgactctt	ccccaaagac	gcaggccggc	tgcgggcgcc	660
ccgacctcgt	ctgaggcccc	tttgtgctca	ctggctgtct	cagaaagggg	tgccacggg	720
aagcgcgtgt	tccttggggc	gcaaggcaag	ggagcccacc	ccaaggtggc	tgagggcaa	780
tggcccaggg	cctctaagga	gtccctgggg	gccgggccc	cctgcagctt	gaggaggaga	840
gccctggctc	tgctcccccg	ggcaggtag	cccacggcag	ggggctcccc	agcagccttg	900
gcaggaagca	gtgaggaagg	ggtgaggatg	aaggcaaggg	ggcctgcggg	gacttgggca	960
aagcccctga	agaactgagt	tcctcgaaaa	ggccggagcc	ctcagccgag	cctcggcctc	1020
cgagcgatgg	aggcgggccc	cctgcggccc	cagggtgcag	ctgtgcatcc	gtccccctcg	1080
ggcctcccc	tgcccccccg	gccaccacac	tctccccctt	ttgcctttga	tcacttgagt	1140
gcgacagctt	gtgcggcctg	agcccagag	accgctgccc	ccctgcgcgc	agcccacagg	1200
gagcgtccac	ctgggcctgg	cctgggcact	catccctccc	ggatgaggcc	tttctagcct	1260
gggcccggcc	gggagcggca	gaccagccc	ctcgcccccc	tccccagtg	aaggtgctgc	1320
ctggtggtct	ggggaagccc	ctggaacagg	gggcgcaggt	cccacacggg	tgctctggcc	1380
tccagctgcc	agggagggcc	gcgctcaggc	cagggtcccc	tccaccagaa	ccgccagggc	1440
cctggggaaa	acctgtctgt	gctaacaggg	ccgctccccg	ggactccacg	gagaggtgcg	1500
agggaccctt	gagcaccac	cgccactaag	gggccagcc	agctcgcggg	tgcaggcagc	1560
cggctgggcg	ctcacatgca	tactgtcttc	tggctttgtg	tgtgcgcctg	ggttggggtg	1620
agcggaggtg	cccgaaggcg	gaagagccca	ccctccactc	ggggacctat	ttcagcaaga	1680
agacggatgg	gactgccggg	catggacaaa	ggaacaggat	gaaccttctg	gaacgcacaa	1740
ggcttccacg	gctgaccggt	cataggaagg	cgcgtctcta	ggccaatcca	ccgtccaccg	1800
tccattcccc	agccctcgag	agggggcagg	atggaccgct	gcagcgtgag	agagctctgg	1860

ggcgcctccca cagggcaaaag tcccagggca ctgacctcag agcccaacca ggccaccggg	1920
gctgggcca ccagggagcc gggggcaggg tcagggtcag ggcccagagt gcgggaaagg	1980
gtggcgtgtt gcttggggcg gcgggcgcgc agacggcccc tcgcaccccc cgacagccct	2040
ggagctgagt gaagcccgcg ggtcaccttg gctggggttg gggctctctg cgaccggcac	2100
cccagctcag gtcacacctg ctgtaccgca gaggggcagg ggttctgagc agggacaggg	2160
tggggcgcgc aggaagcccc cttctctctg aggctgcccc ggccctggag cctctctggg	2220
gcatgccacc cctctcacag acgcctccca ggagcccca ctttctgct gcgtggtgag	2280
ggtgtctctc acccgattcc tggcccctgc aggtcgagtg agtcctgct aagcctgggg	2340
ttggagcagg tgcagggcat caccacacag cagcagaggc tgtggggggc cctgagaggc	2400
gctcccaggt accctcctca gggggctgag cccggggttg acccgggacc tcgcctgccc	2460
caaagccggc gccctcctcc cgcccggccg accagggcca gagaagcagg tgtggggcgg	2520
cacaaacca agtcagcttc cagatcctgc tggggccgcg ttgaaactcg aagccccag	2580
gctgggaggt ctagacaccc ctgccagac cgacagcctg ggccctggctc acagctgcct	2640
ggggggccag ggggtgcacct gccctgtggg tgggggtcag agggcagggg accctcgga	2700
aggtcccca ggggtcaagg tgggcctaag ctccggtgac ctctgggaag tctggggctg	2760
ggttttgttc ccagaggaga gagggccagt agcctcagag gggctgtggc acggtgggaa	2820
ggccccaggt gaccccagag cgtgcgaagc aagccccctt gactgcaaag cgcaaagggc	2880
agaggtgggg tgggagcctc gacccccga gccaggtac acagggggaa gggcgaggga	2940
tccggcaggg gccacaccg ccaccccagg cagcccacaa agcctttggg cccggagccc	3000
cagatggggc cagcccagct ctgggaacag tcttcccaga attcccagc tctgggtacc	3060
aacagggctg cccggccccc agagccctcg ggggggagac ccttcccag ggggatctcc	3120
taagtggcaa ggccctgttg gaggggctgg tgagaggcca ctctggcggg aagaccccca	3180
gccacctgga gccctagcc actgcctgct gcggctccct agggatccag ggccatcaga	3240
gaagctccag cgacactgtt tattttcaaa tgacactttt taagaaaaac agcctcacc	3300
aaatgcttgg ccctgagtct ggaatgtgca gacagacagc tgcccctccc cagagcctgc	3360
acggccctcc ggggtgggga ggagcagggg gcacccctgg gaccgggccg caggctgtca	3420

gggcacggaa cgtgtctctg ggccctgtcc tcaattcccg gtgcccagtg gcccacactt	3480
cccagcagac ccagcagggc cccagcttgt cttggcctgg ccgctgggtcc tgtcacccca	3540
ggcctggagt tctggaagat tctgtctctg ctcccgtgtg cacataccac tccccggggc	3600
agccctgcac ttctgttctt gctggggtcc ctgctgcat ccgtgaggcc tgcagcccg	3660
ctgatcttcc aggtctctct ccgagccccc gcctccagga agccctccag gagagctcag	3720
gagggtcggc tccctgcgcg cagctgtcag acccctgggc ccaccccgcc ggctgctagg	3780
gtccaggttc cccacaagcc ctcgggcaga ggctgggccc ctgggtccct cggagacaac	3840
tggctccgag gccttgccct agacgggttt ccgggagccc gtccccagcg gcacccactg	3900
agttttgaac acttggcgcc acccccacac cccaggcggg gcccaggagg cctcctgggc	3960
agcagacagt ccgtgagggt gccctggggg ggctcctgac ctgggcgctg gccagccct	4020
gggcacagct ttccagatct tgctgcccgc ttctccagg ctgcctcggc ccctcccgcc	4080
tgggggtgcc cagcttttcc tggaggatgc ccacccttgc ccatggtcag ggaggggctg	4140
agaaacccca cctcgtgcct ctgcccggcc tatgccaggg gaaccagggt cctcccgca	4200
ggagggggacc gagtccctga cagcccactg cagaggggag gaggtgcctg gctctgcccc	4260
cagccccacc aaccccgctg cttcctgttt cgcagccac aaagcactaa aggccgcagg	4320
tcctggaaca tcaaagacct gggaagtcca ttgtattgaa ttgagtgtaa atgagcctga	4380
ggcctgtggc ttgcgtttcc cacaattacc gctgcccggg aagggtccg gaaccgacac	4440
agccccagg gcccttgcc catgtgggga gcccaggctg gcctgaagaa gcccataag	4500
gtggacccca ctttgagccc ccacgagagt gggccaagga ccaggtcagg ggctgcccag	4560
gctctgggccc tcctctgcct gccagggtgg ctcctcggg gccagcctg gcctgcagga	4620
ccttcccacg ctgagttccc cagcctggta tgagcgtagt ggacggcagc catgcccagc	4680
actcaggggc ctgagggaca gagcgggaac tccagccccc gggtcctcgg cccctaggat	4740
ccttctaggt ggggaagccc aaggagcag aggggtgaac gcagctgtgt ggggccccag	4800
gctgccgagc agaccctcc tgctccactc ctcgcccgag tgggcgccga gatgccgggg	4860
cagtgccatt tcccaggcgc caccggaggc tcccagagg agtgaggcac gagctgggag	4920
ggagggcggg ggggctgggg aggcagagag cggaggccgg aggccggtga ggaggcccgg	4980
agggggcctg gagtcaatga cccagggatt atcgtgctgg gtctttgcaa agttggctga	5040

gcaaacgccg gagccaaggg tcagggagac gggactggcg gggccccgcg gccccctttc 5100  
ccctttcttg aaaaagcctg tttcccaggt caaatccag ctcatgatcc gccccctttg 5160  
ggactgatgt tcagaggccc agtgggtccca gcacctctgt ccaccgcccc cccacgctc 5220  
ccggggccgc caaccctgt gggctgcgag gtgcgggcac ctctcccttc gaagcaaagc 5280  
cctgccctgc gtgggcagcg tgatttctg cttctctggg gctgcacttt gactgggggtg 5340  
gggggggtgg 5349

<210> 120  
<211> 1593  
<212> DNA  
<213> Pig

<220>  
<221> misc\_feature  
<222> (1551)..(1551)  
<223> Nonspecific nucleotide

<400> 120  
agccctcag cccctccgag cagctgctgg gctcagcggg ctgcccccc gatgtgcggc 60  
cctccataat caatcatgga gggccgggcc cggggggggc gggccgacct gtcagccagc 120  
tccaagggca gggacagctg ctgttccgga gggttcccag gggccagccc caccagacag 180  
cggcctcggc cccccctccc cgaggggcac cccacggag ggccagacc ggagggactc 240  
ggggcccaga ggccagggca agagtgaagg cagcgccggt gggagcggcg gtcagcgggg 300  
tccaggcttc agttcccaag gagccccatg cctgagccc gcactgagcc ctgtgcagcc 360  
tgtgggtgcc gccgaggccc gccacccgc ccccaccag cctggggtcg aaggaggag 420  
ggggtggcct gacggatggt aacagctgct cccccacct cgccggcgtg gacagggctc 480  
gcttctctg cccgagcccc cggtgcccc atccgtcacg gccacccag gactgtgcgt 540  
ccagcctccc tcctcctaa tcccccgca ttttcgaat tctcgggcca ctgctgcttc 600  
ctctcaaat tcctggcccc cctgccccca tccccgcat gggaaagggc cgcatgcca 660  
ggacattgc tcgtctcggc cgggcggggg gaggagcagc tggctgggcc cggcagctgt 720  
gaggtgcggg ggtgccaggg agaagggccc agattaggg gcgtcatggg aaagctggga 780  
gggaacgcta cccagagccc ctctgccgc agcctgtgct gctccctctc cgcatttctg 840



gcctctgagt gctccctgga gggaagggac cactgtgtcc tgccggcctc tggctctgcc 900  
 aggaatgtcc atctgtccgg gccgggttac ctggctcaga gcgtgggtac cagctcatcc 960  
 agccctgacg cctgctctcg ggaacagtgg atgggccagg cgcctccgctc acaccccgca 1020  
 gctgggctcc acagacgggc ccgggatggc cacggaggtg gggggcggcc ccagggcgag 1080  
 gctccctcct ggaagggcta gagtgtgggc tgcgcggaga gggaggccgg acggccaggc 1140  
 cagggtgcagc ccggggcagg tgctgggtgg ggctgtgacc cacgtgtgca gctcaagggc 1200  
 ccaggagccc caggacaga gcctcagga cagacctca gagccacagc aggaagcctg 1260  
 gtggcagtag ctggcggggc cgtgggtgctc tcggccctgc agacagaggc agaggcaggc 1320  
 tccctgctga tgacaggggc tttctctgtc ccctgggggg cggagggggc ccgaccatgg 1380  
 acccggggcc tcctctcgca cgattccag gccagcctgg tctcaggcag tccaaggttg 1440  
 cacaatggtc tccatcgtcc agagttgcag agccagcact ctccactgg acggcggccc 1500  
 ggggtgggct gcaccgccgc tcagggtcga gggccgcggc cggccagccc nccgcaggcc 1560  
 ttgacctgt ctcttataca catctcaacc ctg 1593

<210> 121  
 <211> 831  
 <212> DNA  
 <213> Pig

<400> 121  
 tgagatgtgt ataagagaca ggccttgacc ctgggcctgg ctgagctgcg cgcctcctc 60  
 cttgcagctc cgcctcgacc ccatccatca gccattttcc tacccttcct gtaataaaaa 120  
 acccgaagcg gcgtggcccc gtgtccgctg gggtgactgc ggctgcctg ctggtggctc 180  
 ccacctgggc ccggccccct gaaaacacac acccggcgat ggcttgcccg gggccctggt 240  
 ggagggggcg ggggcctcgc ctgcctcttg tctgaaattt tcggtccac atgccccgac 300  
 tcctctcccg gccaccctg caggcccggc cggtgccccg gccactttcc cgaaggacgg 360  
 actcagcatt tcccagggca cctgctgatg gtgccagac cccgggggcc ttcccgccgg 420  
 gcgcggcccc acgtcgcccc tccagtggcc acagcgggcc tgggccaagg ctgggagttc 480  
 tgcacgggcc tgggggagga aggcggggga gaggggacag tctcctggcg gggacgaggg 540  
 tgggggcagc aggtggggag ttcccacagc cggggcagcg ggacgccgct tggctgcct 600

gggtctcagc cggggacagt gcccaccagg agagagacgg cagacagtac agcccacccg 660  
 ttttatatcc tctcaggcgg tctgtgcttt attggggtaa atatgcagga catagaaact 720  
 ctgccactgg accccttggc cgggggacac agcagcggca ttgcatgctt tctgggtgca 780  
 gcgcagccag caccaccggc cagagcacc cctcttcccg atcaaccgga c 831

<210> 122  
 <211> 4636  
 <212> DNA  
 <213> Pig

<400> 122  
 ctctgggcta gcaccgtggg ggctttgcc a gactgggta cccccggag 60  
 ccagagggc ggtgaatggg aggcagagcc catcctggga atggaccaga agaaaggag 120  
 cgggggtggg ggaaggggca tcagatcctg gtccttcctt gtcgctgag gtccctctgc 180  
 caccactccc cgaagctgat ctggagcaca cgcgtcgta aagccgcat cgaggcccca 240  
 cttctgacag acggaagggg gcagagtgcc ttcctcaccg gcctcgccct gggaaggccc 300  
 ctccctgcag ccaggaagc cagcagcagg tgacagagcc aggggcccag ggccccaggg 360  
 acgggctcgc gcgcccagc cgggggtccc ttggcgctcc catcctctcg tcttgagcc 420  
 ctctgggtg accacaggaa tgtgcaaggc ggcagccggg tggcgccggg gaggcgggtg 480  
 ggaggcgggc ggggtggcct cttcacgggc gggcctgaga gatgggcgcc cgtccggccc 540  
 tggcgatcgc gtctccgct ctctaccac tgagcaaaga cacacgaaat gaagctcgaa 600  
 cgagcacagc caaagaacgg ccgtttctgt cctttcttct taatcccttt ggcttagggt 660  
 ttccggcct ggacagcctg cccaagggca catgggcata cgtccgggga cattcaggca 720  
 gtgaccaatc ccaggccacc caggctgtgc cctgcgtcgt gggccatttc ccagccggcc 780  
 agagatggag cagccactgc ggggtccccg gtctcggtga gacagtcaag gatggacctt 840  
 ggatggagac cggcgtgagg ccatgtccgt ggggtgaagga ggcgtgcagg ccgtgctggg 900  
 ggacatgggt gctgtccct cggccaaacc atgaaaagca gccctctccc ccaaccccca 960  
 gcaccaacc ggagaccacc ctggccggga gccagcacg gccaccgtca cgtctcggtc 1020  
 gtccagcttg ggacaggtca gttcccagat gtccaggctg gagctggtcc ttgaagatcc 1080  
 taggggtcca gccagcaca ggaggccag gtgagagccc cctgtggttc taaggatgca 1140

accagggggcc ggcgggggtgc ctgccctaga gggggtaact cggccccctg gggaccagtc	1200
accccaggag gtccccagag cccagctcgg agggccacag gtgcccagag tcccacctgg	1260
ggaaggctgc ccctcctgcc agcccccgag cggggcccct ggcgcccgcg tccagccgcg	1320
accccgggga gatattcacc ccctgcccc gtgaatcagg aggccccgag cccatgtttt	1380
cagtcctttt cctcccatcc cagcccccca ggagaagagg tgctgaactg ggtcccctgg	1440
aggctcctga gcccagaac agtgccctct gagcagacgg gcactctcag accagctcac	1500
gctggacaag tcagctcctg cctgccgcct gatggggcct tgggagaagc agacatggtg	1560
aggaaaaggc ccctgtgccc ttcaccctaa ttccccagcc ccaagtccca ctgggttgcc	1620
agcttcaacc taagcaaata attcgtgccc tctaaacaaa cgcgcgggaa tcccacctgc	1680
ccttcccccg ccgcccccc caccctggc cttgacctcc aaaagcactt gagggggctt	1740
tctccagaca ccctccaacc cgcacccat gaagaagggg tgatggggct gttacccaa	1800
caagcaagag aacgaagccc agagaggagt tggcgtggac agcaggggtc agggcccttt	1860
gccccgaggg cagggctggt gccacctggg tcaggcggca ggcctggaa aagcaccgga	1920
aatgagcaca cctgggtctc tagaaggttc ttccagacct ctgggggctg agtcatttca	1980
acactcctgg gccgggcagg gcttcttctt ggcgccgagg gacaaggctc ccttcgtccg	2040
gggggtacgg cccttgacc cctgtcccc gcacccacc ctcgcctgg tgagggccgc	2100
ggccagctct ggacacagat ccctcagagc ccttctctcc tccctgctcc ctgctctcc	2160
caagatgccc cggcctccag gtggggcagc caggcggcag aatgtggtcc aggcctctcg	2220
gccccacca caccacctg ctctgccctg acagcctcca agacgcaggc acgtcgtgc	2280
gttctgcgtc ctgtctctc atggcacaaa acggtgccc cctagcttcc ccagagaag	2340
ggagatcgtg ctccccggac ggacctgct ctgcctgtcc tcccggccg ccttcagggc	2400
ctctcccaa gggtgccgc gaggaggccc tcgcctccg ccacggggc tccatctcc	2460
cgagcccgac aggcctccgc ctggtggtcc gacctcttc ccaaggcccc gccatcctc	2520
ctcgcgtcc cccaaacct gctcttttc ccagcgcct tgtccccacg gaagacctc	2580
caccgtgcc attacagct ctcgcccac cctcccagcc accccccctt cccatcctc	2640
ctggaagctc ccacttctt ccgctctcc acggcagcag agggtcagca gctcaggggt	2700

cctggggccg tggagatggc ctgcccgggg gtctcgctga ccgcctccta cggaagctgt 2760  
 gccgggggggt ggggggtgtct ctgcccgaac ggctggagga cgagccacat cccagggcag 2820  
 ccggaacctg cgtcctggtc tgagacggag aggctgggtg caggtggctg aggggcctgc 2880  
 acacagcttg gcctggggtc ccctaggtga caacactggc tgaacactca ttgctgctcc 2940  
 ccttcagggt tgaccctggg gtccccgtgt ggcctcagg gcacacgggg gcccaccag 3000  
 gcctcacaga accccagtgg gactgcaccc agggcccaca gaagtgcggg ggcactgggg 3060  
 gtccagaaac aaccccacaa ccaggccaag gtggccaagg ccttactcga gcggggctgc 3120  
 ccgtcccaag agactctggc cagtctccg gatccagctt cccggggccg ggccgcccgc 3180  
 tgggtccag gcggttctgg ggggccctcc cccgggggtt cgcctccgc tctcagcagc 3240  
 aggaagagga gcgcggccag cggatgggga gaagaggcg cctgggcat cttgctcccc 3300  
 ctgggacttg aggagggctt cgggccgggc aggcgggacc gggagccaca gagaccctgg 3360  
 aggaggcagc atggcgggga ggtgaccggg gaagagggcc gtgtcccagg ctacagccc 3420  
 ggctggccg cccggccctc gggaggcgtg ccgctgaccg cctggccggg aggtttgctg 3480  
 cgtgtgggggt ttgcagaaag tgctgagctg ctgagccac aggcaggct cagaggggac 3540  
 aggaaggagg ttgctgcca gcctcgggca ctgctgacc atctccggt tccaggcac 3600  
 cagagccacc taatctgcg gctctgtgcc caggacagg cttgcctgat ctctcaaggc 3660  
 cgggcgtcc gccttcctg ggagagggtt aaacatccag cccagccag catctcgggc 3720  
 aggttcctg cccccccgc tcgtgcctc tctgagacc tggtcggcac acctttcct 3780  
 tgagaggagg aggaggagga aagcggatgg aaccagtac cctgcagccc ctgagggcac 3840  
 ctccccagt gccccgccc gcccgcgtc ctccgcccc agttctcac gccccagtc 3900  
 tgatggaggg agggcgacct ccgggtccc tggctccgc cggctccga agacagggcc 3960  
 gctcggctgg ggctgcagg aggggcccga gacgcaggag agcagcccgg aggcaaacc 4020  
 cgcggtctt ccagaaggag gcctggcagg gggagggggg tgccaccact gctgtccctc 4080  
 tcgtgccaca gtggagggtg tgggtgggca gtgccgggt ggaagtga gaaagacct 4140  
 ggaccgtgg gctgggccgc cacgggggag cgggtctgt caggaccct gggggaggga 4200  
 ggcgaagggc tggggcagag gccggatcac ttccagattt gctgtgggac caagggccg 4260  
 acctcgggt gacttcttt gtgtgctggc cacaggggg cccggcgag gtcacacgga 4320

agggggcttc ggacctggcc taacaagccc actcccgagg aagatgcaag gggaggcaga	4380
cggaagggcc gaagggggcg atcgggggac accgcggcag ggccggggca gagaaggag	4440
gcagagggca gagaaggag gcagagggca gagaaggag gcagaggggc cacatgcttg	4500
gagggccagg gaggagcggg aacggcgctc ggcgccagc gccgaatcag gcccgtcagg	4560
cggaggggtgc gtggacctgc ctggccttca cgagcacagt cagcaggctg tctcttatac	4620
acatctcaac catcat	4636

<210> 123  
 <211> 482  
 <212> DNA  
 <213> Pig

<400> 123	
agcaatgggg ccgtgacctt aggaggcagg cccaggctcag tgggggtgacc tctcgtggcc	60
ccgatgtttg gaaatcccca aatcaaaatg acccatccga caagcttgca tgcctgcagg	120
tcgactctag aggatccccg ggtaccgagc tcgaattcgc cctatagtga gtcgtattac	180
aattcactgg ccgtcgtttt acaacgctgt gactgggaaa accctggcgt taccctactt	240
aatgccttg cagcacatcc ccctttcgcc agctggcgta atagcgaaga ggcccgcacc	300
gategccctt cccaacagtt gcgcagcctg aatggcgaat ggcgctgat gcggtatttt	360
ctccttacgc atctgtgcgg tatttcacac cgcataatgg gcactctcag tacaatctgc	420
tctgatgccg catagttaag ccagccccga caccgcgcaa caccgctga cgcgaacccc	480
tt	482